

Comprehensive Assessment of Long-Term Effects
of Reducing Intake of Energy (CALERIE)
Laboratory for Clinical Biochemistry Research, University of Vermont
Manual of Operations
Version 5: 04232009

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SUMMARY OF CHANGES FROM VERSION 4 TO 5

In addition to typographical and spelling errors, the following substantive changes were made to Version 4 to create Version 5.

Quality Control Review Approval

- **Appendix E:** Additional QC Data review signature template added for use when reviewing serum set, serum high/low, and LCBR performed assay control data. To be signed by PI or Lab Coordinator.
- **Sections IX and X:** Appropriate updates have been made to include instructions for use of the signature approval templates.

New Database Functionality

- **Section III:** Additional instructions added to section on label printing to incorporate new options available.
- Updated screen representations to reflect current database appearance throughout MOP.

Annual visit Blood Collection Kits no longer gender specific

Throughout the MOP several changes have been made regarding gender assignment to the Baseline, 12 Month and 24 Month blood collection kits. Since we are no longer testing progesterone levels in female participants, there is no difference between the male and female kit components.

- **Section III A:** Kit component labels for annual visit kits are no longer 2 separate files distinguishing male and female. One label set not specific to sex has replaced these two separate files.
- **Section III B:** Additional tubes for sex hormone collection have been removed from annual visit kit components.
- **Appendix C:** Changes were made to aliquot #52 as a result of deletion of progesterone testing for female participants. Aliquot 52 is now sent to FAHC for male sex hormone panel only. Aliquot #52 for female participants is now stored in LCBR assay box. Aliquotting Guide has been updated accordingly
- **Appendix A:** The Schematics of LCBR Aliquotting Scheme has been updated accordingly.

Insulin Testing Method Change

Initial testing of CALERIE population via FAHC method yielded a substantial amount of low unreadable results. Change made to insulin assay method from FAHC chemiluminescent assay via the Immulite analyzer to Alpco Ultra-sensitive EIA done at LCBR.

- **Section VI:** LCBR and FAHC Assay Methods updated accordingly.
- **Appendix A:** Schematic of LCBR Aliquotting updated accordingly.
- **Appendix B:** Testing and Repository Box Maps updated accordingly.

- **Appendix C:** Aliquotting Guide updated accordingly.

Additional Miscellaneous Changes:

Section III B through E: All Kit preparation instructions updated to reflect new Ziploc bag sizes available

SUMMARY OF CHANGES FROM VERSION 3 TO 4

In addition to typographical and spelling errors, the following substantive changes were made to Version 3 to create Version 4.

Edits made in response to CALERIE QC Site Monitoring Visit

On June 11, 2008 the CALERIE Core Lab was visited by Kai Research, Inc. QC inspector, Sally Quataert as part of an NIA initiated monitoring program for CALERIE central facilities. The following sections of the CALERIE Core Lab MOP have been revised in response to observations/recommendations made during this site visit:

- Details regarding who is responsible for all duties assigned in this MOP are now addressed throughout the MOP.
- **Section III F:** Disposal instruction for prepared 10% sodium metabisulfite included.
- **Section IV C:** Step 13 added-instructions on how to correct an error found in form entry verification steps.
- **Section IV F:** Additional instruction added: “Return samples immediately to -80C freezer once scanning is complete.”
- **Section IV G:** Additional details added regarding whom, when and how samples are sent to FAHC.
- **Section VI:** FAHC box description table deleted.
- **Section VI:** Added FAHC Method Summaries and LCBR Method Summaries.
- **Appendix C:** Two major updates to Aliquotting Guide-
 - Increased volume of CPEP aliquots (75, 08, 09, 12 & 13) from 300uL to 400uL serum. (requested by Mayo Clinic)
 - OGTT tubes 38-40 now aliquotted into 4 vials- 1 for SGL, 1 for CPEP, 1 for Insulin, and 1 for OGTT repository.
- **Appendix D:** Addition of table listing all CALERIE supply ordering information including vendor and catalog #'s.
- **Appendix E:** Addition of CALERIE Data Review and Approval Signature Templates

SUMMARY OF CHANGES FROM VERSION 2 TO 3

In addition to typographical and spelling errors, the following substantive changes were made to Version 2 to create Version 3.

Sex Hormones in Women

The CALERIE Phase 2 Protocol v14 03-27-08 deleted the measurement of sex hormones in women. The following sections of the CALERIE Core Lab MOP have been revised in response to this study protocol change:

- **Section II:** The baseline, 12 Month and 24 Month visit summaries have been revised accordingly.
- **Section III B:** The Female Sex Hormone and Off-Cycle Female Sex Hormone Collection kit preparation instructions have been removed.
- **Section IV:** Instructions regarding the receipt of off-cycle female hormone and annual visit female sex hormone samples have been removed where necessary.
- **Section VI:** Progesterone removed from CALERIE Core Lab Assay Methods
- **Appendix A:** Removed Off-cycle sample processing path schematic

Additional Instructions new to this version

- **Section IX:** “Import of Assay Results to the CALERIE Sample Management Database” was added to the MOP
- **Section III A:** Instructions regarding blood draw kit expiration date determination and assignment were added. Instructions on creation of serum set and serum high and low control labels were included.
- **Section III E:** Inventory and Tracking of Kits instructions were added to MOP
- **Section IV F:** Aliquotting of Serum High Controls, Low Controls and Control Sets instructions were added to the MOP.
- **Section IV G:** An example of a FAHC Test Request Report was included.
- **Section X C:** Added instructions on Uploading and Transmitting a Batch to Duke.

SUMMARY OF CHANGES FROM VERSION 1 TO 2

In addition to typographical and spelling errors, the following substantive changes were made to Version 1 to create Version 2.

Addition of Annual visit Insulin and CPEP to testing performed on OGTT samples

- Appendix C: Changes to the aliquotting guide to include: aliquots 18, 19, 98 and 99 are no longer being reserved for Bruce Kristal ancillary study. These vials are designated for C-Peptide testing now. New aliquots 38-41 have been added to the aliquotting guide. These vials are designated for SGL and Insulin assay.

I. STUDY OVERVIEW AND DESCRIPTION

The overall aim of CALERIE Phase 2 is to test the hypothesis that two years of sustained caloric restriction (CR), involving a reduction in energy intake to 75% of baseline (25% CR), in healthy men and women aged 25 to 45, will result in the same adaptive changes that occur in rodents subjected to CR. Particular emphasis on the adaptive responses thought to be involved in slowing the aging process and protecting against age-related disease processes. Primary outcomes include core body temperature and resting metabolic rate. Secondary outcomes include triiodothyronine and catecholamines (as potential mediators of the predicted metabolic adaptation), and plasma concentrations of TNF- α (because inflammation is one of the adaptive responses suggested as a mediator of the salutary effects of CR on the aging process in rodents). An important secondary aim is to identify potential adverse effects of CR in humans. A number of exploratory aims will be assessed to evaluate the effect of CR on body composition, serum hormones, plasma growth factor concentrations, serum lipid and lipoprotein levels, skeletal muscle, adipose tissue and psychological factors. Consistency between the two sexes and across levels of body composition will be explored. In addition, biological samples will be stored in a biosample repository for future analysis.

Basic Study Design: The study will be conducted as a multi-center, parallel-group, randomized, controlled trial (RCT). A sample of 250 participants will be enrolled, and assigned to either the CR intervention or an *ad libitum* (AL) control group. A 2:1 allocation ratio in favor of the CR intervention will be applied in order to maximize the number of subjects receiving the intervention of greater scientific interest. Participants in both treatment arms will be followed over a period of 24 months. A comprehensive set of evaluations will be performed prior to initiating the intervention, with follow-up evaluations at Months 1, 3, 6, 9, 12, 18 and 24 after randomization. It is expected that 10% of study subjects will drop-out in each of the two follow-up years, so that a sample of approximately 200 subjects is expected to complete the study.

The Laboratory for Clinical Biochemistry Research (LCBR) at the University of Vermont is the Central Biochemistry Lab for this project. This role includes:

- Oversee specimen collection at three sites and shipment of samples from the clinical sites to LCBR.
- Create and maintain the CALERIE Phase 2 Biosample Repository.
- Measure markers of inflammation, hormone levels, growth factors, and other analytes as specified.
- Manage shipping of samples (e.g., tissue biopsies) to alternate testing sites as specified.
- Provide QA/QC assurances on the above.

II. SUMMARY OF SAMPLE COLLECTION

Timepoint	Visit#	Summary	Collection method	Comment
BL	7	'Hot Box' for catecholamines (3 collections)	IV	
		Fasting: All measurements & blood/urine archive	IV	Plus 24H urine
		30 min OGTT, CPEP, Insulin	IV	
		60 min OGTT, CPEP, Insulin	IV	
		90 min OGTT, CPEP, Insulin	IV	
		120 min OGTT, CPEP, Insulin	IV	Plus tissue kits
3M	1	Fasting: blood/urine archive	Venipuncture	EDTA/Serum
6M	5	Fasting: blood/urine archive & bone measurements	Venipuncture	EDTA/Serum
12M	4	'Hot Box' for catecholamines 3 collections	IV	
		Fasting: All measurements & blood/urine archive	IV	Plus 24H urine
		30 min OGTT, CPEP, Insulin	IV	
		60 min OGTT, CPEP, Insulin	IV	
		90 min OGTT, CPEP, Insulin	IV	
		120 min OGTT, CPEP, Insulin	IV	Plus tissue kits
18M	1	Fasting: blood/urine archive plus AB response	Venipuncture	EDTA/Serum
24M	4	'Hot Box' for catecholamines 3 collections	IV	
		Fasting: All measurements & blood/urine archive	IV	Plus 24H urine
		30 min OGTT, CPEP, Insulin	IV	
		60 min OGTT, CPEP, Insulin	IV	
		90 min OGTT, CPEP, Insulin	IV	
		120 min OGTT, CPEP, Insulin	IV	Plus tissue kits
17M, 23M		Antibody response	Venipuncture	No repository
Unscheduled		Sex hormones for amenorrhea (LH, FSH, Estradiol)	Venipuncture	No repository

III. BIOSPECIMEN KIT PREPARATION

Introduction

The Laboratory for Clinical Biochemistry Research is responsible for supplying the CALERIE study field centers with supplies needed for the blood draw and urine collection sections of participant visits throughout the 24 month exam period. Kits are specific to visits and CALERIE field centers. The Baseline, 12M and 24M visits are also specific to male or female. The following is a protocol for assembly of the CALERIE blood and urine collection kits needed at each of the nine potential visits that occur over the duration of the study. CALERIE Core Lab certified Research Laboratory Technicians will complete the assembly of kits as requested by the sites (Steps A. through D. to follow).

A. Labels

Tube, cryovial, and form labels

Labels will be used on forms, draw tubes, cryovials, transfer tubes and urine collection containers. These Sample ID numbers will be different from the participants CALERIE ID number and care must be taken to correctly identify the CALERIE ID number with the Sample ID number. These unique labels will allow for each sample to be tracked individually throughout the study.

Example:



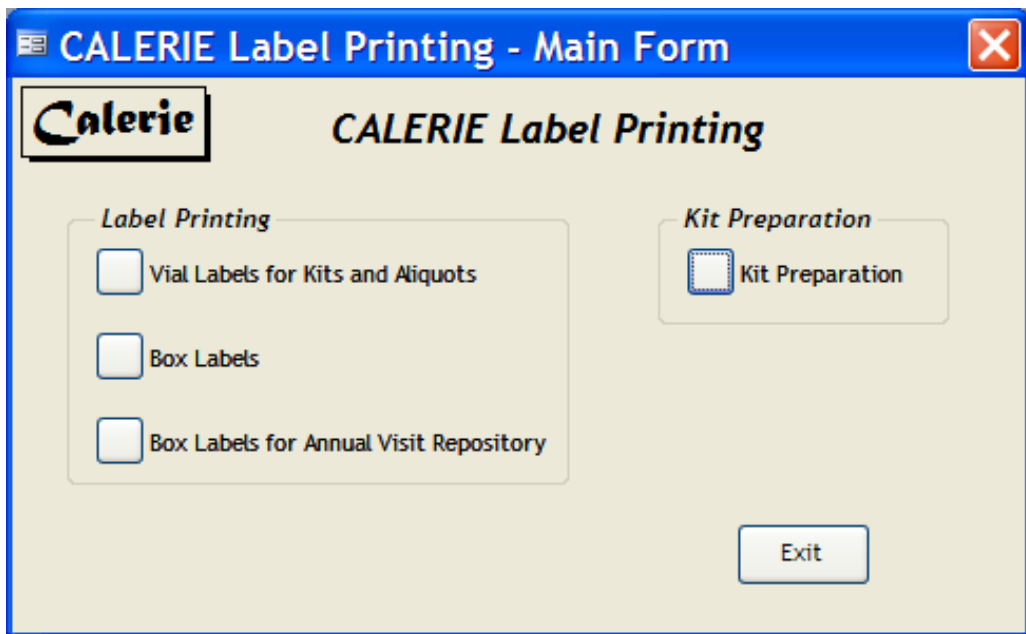
The format of the labels will be: YYYY-00-ZZ ex. 1234-00-02
 Labels for forms will be YYYY-00 (sample-visit) – no tube #

Site #	Sample #	Visit	Tube/cryo #	comments
X	YYY	00	ZZ	
1= Pennington 2= Tufts 3= Wash U		03		
		06		
		12		
		17		
		18		
		23		
		24		
		99		99=Unscheduled Visit
		80		80=Baseline Tissue
		82		82=12M Tissue
		84		84=24M Tissue

Adding new kits to inventory

The following steps are to be completed by a CALERIE Core lab certified research laboratory technician.

1 The label-printing database is called 'CALERIELabelPrinting.adp' and located in folder <\\Med15\\Shared\\Groups\\LCBR\\Databases\\CALERIE>. note: the *kit preparation* table accessed from the label-printing database can also be accessed from the *CALERIE Sample Management Main Screen*. Laboratory technicians can only perform this action on computers that have the BarTender software installed. Select *Kit Preparation* on the main form. Kits must be added to the kit inventory before label sets can be printed.



CALERIE Label Printing - Main Form

Calerie **CALERIE Label Printing**

Label Printing

☐ Vial Labels for Kits and Aliquots

☐ Box Labels

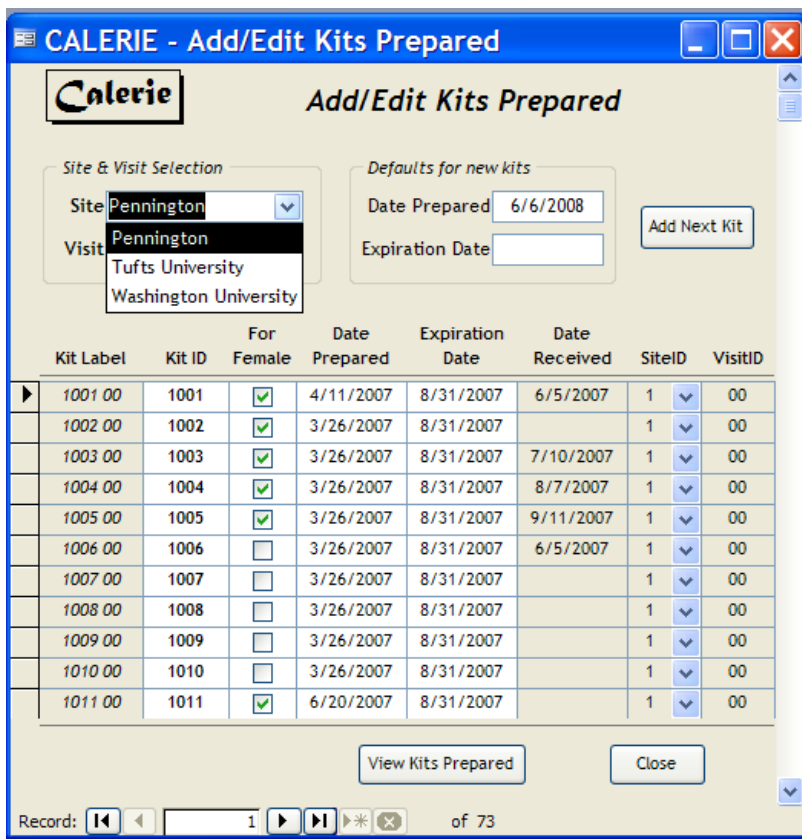
☐ Box Labels for Annual Visit Repository

Kit Preparation

☐ Kit Preparation

Exit

2.) The Add/Edit Kits Prepared Form will appear. Select the *Site* and *Visit* from the drop down menus provided. The *Date Prepared* will default to the current date. Edit this if necessary. If you know what your kit expiration date will be you may enter it at this time. If not, you will need to edit this field after kits are prepared. (See Section E. *Inventory and Tracking of Kits*)



CALERIE - Add/Edit Kits Prepared

Calerie **Add/Edit Kits Prepared**

Site & Visit Selection

Site: Pennington

Visit: Pennington, Tufts University, Washington University

Defaults for new kits

Date Prepared: 6/6/2008

Expiration Date:

Add Next Kit

Kit Label	Kit ID	For Female	Date Prepared	Expiration Date	Date Received	SiteID	VisitID
1001 00	1001	<input checked="" type="checkbox"/>	4/11/2007	8/31/2007	6/5/2007	1	00
1002 00	1002	<input checked="" type="checkbox"/>	3/26/2007	8/31/2007		1	00
1003 00	1003	<input checked="" type="checkbox"/>	3/26/2007	8/31/2007	7/10/2007	1	00
1004 00	1004	<input checked="" type="checkbox"/>	3/26/2007	8/31/2007	8/7/2007	1	00
1005 00	1005	<input checked="" type="checkbox"/>	3/26/2007	8/31/2007	9/11/2007	1	00
1006 00	1006	<input type="checkbox"/>	3/26/2007	8/31/2007	6/5/2007	1	00
1007 00	1007	<input type="checkbox"/>	3/26/2007	8/31/2007		1	00
1008 00	1008	<input type="checkbox"/>	3/26/2007	8/31/2007		1	00
1009 00	1009	<input type="checkbox"/>	3/26/2007	8/31/2007		1	00
1010 00	1010	<input type="checkbox"/>	3/26/2007	8/31/2007		1	00
1011 00	1011	<input checked="" type="checkbox"/>	6/20/2007	8/31/2007		1	00

View Kits Prepared

Close

Record: 1 of 73

3.) Click the *Add New Kit* button on the right of this screen. A form will appear showing only the next kit id available. Click the “for female box” if applicable. If you have more kits to prepare, repeat this step changing the site and visit where appropriate.

CALERIE - Add/Edit Kits Prepared

CALERIE *Add/Edit Kits Prepared*

Site & Visit Selection

Site: Pennington

Visit: Baseline

Defaults for new kits

Date Prepared: 5/25/2008

Expiration Date:

Add Next Kit

Kit Label	Kit ID	For Female	Date Prepared	Expiration Date	Date Received	SiteID	VisitID
1074 00	1074	<input type="checkbox"/>	5/25/2008			1	00

View Kits Prepared Close

Record: 74 of 74

Printing a new tube, cryovial, and form label set

The following steps are to be completed by a CALERIE Core lab certified research laboratory technician.

1. On the main menu, press the ‘*Vial Labels for Kits and Aliquots*’ button. On the dialog that pops up, you need to make a number of selections.
2. Select the label printer. You'll need the 5-across labels loaded onto the Zebra label printer.
3. Select the Label Group. You'll want to select *Kit labels* here.
4. Select which visit you want labels for.
5. Select the appropriate Site from the listbox.

6. Select which labels you want. By default, the 'Print all labels shown below' option is selected, which will print the full set of labels shown in the Label Selection listbox. But you can clear the checkbox and select individual labels if you wish.
7. Specify the *Kit ID Range*. Note that once you have selected a site, the next kit in the inventory will automatically appear in the Kit ID Range box. Be sure to change this range as needed.
8. Then press the Print button. You will be prompted to confirm that correct labels are loaded into the label printer.

Kit component labels

Each kit will be labeled on the outside with the visit, Participant ID, site where the visit will occur and an expiration date of the kit. For the baseline, 12M and 24M visits the individual draw specific Ziplocs that make up the Kit will also have their own labels. These draw specific labels will include the participant ID, site, the specific collection to which the supplies pertain and a spot to record the expiration dates of the kits.

The expiration date of each kit is determined by the draw tubes included in the kits. Review tubes included and select the tube whose manufacturer expiration date occurs first. The expiration date of the entire kit is the expiration date of the tube to expire first within the kit. For example: the serum tube in a 3 Month kit expires Dec. 2008 and the EDTA tube expires Sept. 2008. The kit expiration date is the EDTA expiration date of September 30, 2008. If only the month is given on a tube expiration date record the last day of that month as the kit expiration date.

Word document templates for these labels are stored in:

<\\Med15\Shared\Groups\LCBR\Databases\CALERIE\Labels>

Research technicians will highlight the appropriate kit label info and print labels from Word onto Avery 5162 white mailing labels.

Serum High and Low Control Labels

Each Month a Serum high and Low control will be included in the samples to FAHC for each test performed at FAHC. (See Section IV. F for aliquotting procedure) The labels for the aliquot cryovials are printed from the bartender file *CALERIE-SerumControl labels.btw* located in:

<\\Med15\Shared\Groups\LCBR\Databases\CALERIE\Labels>

Research technicians are to double click on this file to open the program. Print 1 set of labels (n=16 labels) and print to the local Zebra printer loaded with 5 across labels.

Serum Set Control Labels

Every 3 Months a Serum Control set , 20 normal serum samples from unique donors, will be created by the CALERIE Core Lab research technicians for each test performed at FAHC. (See Section IV. F for aliquotting procedure) The labels for the serum set aliquot cryovials are printed from the bartender file *CALERIE-SerumSet Labels.btw* located in:

<\\Med15\\Shared\\Groups\\LCBR\\Databases\\CALERIE\\Labels>

Double Click on this file to open the program. Print 1 set of labels (n=140 labels) and print to the local Zebra printer loaded with 5 across labels.

B. Annual (baseline, 12M, and 24M) visit kit preparation

All kit preparation is to be completed by a CALERIE Core lab certified research laboratory technician.

Kits are also specific to site and visit. Be sure to use labels with appropriate site and visit ID.

Supplies needed prior to assembly:

- Baseline, 12M or 24M Label Set –next available participant ID needed at site (see printing label set instructions above)
- Forms – “Hot Box” draw P/P Form (1), Fasting draw P/P forms (2), OGTT draw P/P form (1), Urine Collection P/P form (1)
- Ziploc bags-1-Gallon (2), sandwich (2), pint (4)
- Draw tubes- 4mL Serum (4), 10mL Serum (4), 10mL EDTA (2), 2.7mL Citrate (1), and 2.5mL Paxgene (2)
- Transfer Tubes (16) - 10mL white-capped Simport tubes
- OGTT tubes (4) – 4mL Sarstedt tubes (FAHC tubes)
- 50mL Corning polypropylene tubes (2)
- Cryovials 1.5mL skirted with color-coded caps – red-capped (3), purple-capped (3), blue-capped (2)
- 250mL Absorbent Strips (1)
- Kit Content Labels (6*) – Labels specific to entire kit and 5* draw specific components within kit.

Catecholamine/Hot-Box Draw:

1. Take out supplies needed for hot box draw:
 - 1 - One sandwich-size Ziploc bag
 - 2 - 10mL Transfer tubes
 - “Hot-box” draw P/P form (1)
2. Affix appropriate labels to the transfer tube (20 and 21) and a form label to the P/P form.
3. Affix kit component label to the outside of the 1 pint Ziploc.
4. Fold P/P form and put form and both transfer tubes into the 1 pint Ziploc.
5. Put sealed Ziploc into the 1 Gallon Ziploc which will hold the entire kit.

Fasting Draw:

1. Take out supplies needed for the fasting draw:
 - 1 - One gallon Ziploc bag
 - 2 – snack-size Ziploc bags
 - 1 - pint Ziploc bag
 - 3 - red-capped cryovials (cryo #22, 23, and 24)
 - 3 - purple-capped cryovials (cryo #31,32, and 33)
 - 2 - blue-capped cryovials (cryo #29 and 30)
 - 8 - 10mL transfer tubes (tube #25, 26, 27, 28, 34, 35, 36, and 37)
 - 4 - 10mL Serum draw tubes (draw tubes #3, 4, 5 and 6)
 - 2 - 10mL EDTA draw tubes (draw tubes #8 and 9)
 - 1 - 2.7mL Citrate draw tubes (draw tubes #7)
 - 2 - 2.5mL PAXgene draw tubes (draw tubes #10 and 11)
 - 2 - 50mL Corning polypropylene tubes
 - 1 - Fasting draw phlebotomy form
 - 1 - Fasting draw processing form
2. Affix appropriate labels to the draw tubes, cryovials, and transfer tubes (see step 1 for cryo and tube #'s) and a form label to the P/P forms.
3. Put all blood draw tubes in one snack-size Ziploc bag. Put cryovials and transfer tubes into the pint Ziploc bag.
4. Put each labeled PAXgene tube into a 50mL Corning tube. Put both Paxgene tubes into the snack-size Ziploc bag.
5. Affix kit component label to the outside of the 1 gallon Ziploc.
6. Fold P/P forms in half and put forms, both snack-size Ziplocs, and the pint Ziploc into the 1 gallon Ziploc.
7. Put sealed Ziploc into the 1 Gallon Ziploc with the “Hot-box” draw supplies.

OGTT Draw:

1. Take out supplies needed for OGTT draw:
 - 1 - One pint Ziploc bag
 - 4 - 4mL Serum draw tubes (draw tube #'s 12, 13, 14, and 15)
 - 4 - OGTT tubes (tube #'s 38, 39, 40, and 41)
 - OGTT draw P/P form (1)
2. Affix appropriate labels to the draw tubes, and OGTT tubes (see step 1 for cryo and tube #'s) and a form label to the P/P form.
3. Put all blood draw tubes and OGTT tubes into pint Ziploc bag.
4. Affix kit component label to the outside of the pint Ziploc.
5. Fold P/P form and put form into the pint Ziploc.
6. Put sealed pint Ziploc into the 1 gallon Ziploc with the fasting and hot-box draw supplies.

24 Hr Urine Collection:

1. Take out supplies needed for 24 Hr Urine collection:
 - 6 - 10mL transfer tubes (tube #'s 43, 44, 45, 46, 47, and 48)
 - 1 - pint Ziploc bag
 - 24 Hr Urine Collection and Processing form (1)
2. Affix appropriate labels to the transfer tubes (see step 1 for cryo and tube #'s) and a form label to the P/P form.
3. Affix kit component label to the outside of the pint Ziploc.
4. Fold P/P form and put form and transfer tubes into the pint Ziploc.
5. Put sealed pint Ziploc into the 1 gallon Ziploc with all the other visit supplies.
6. Affix Kit contents label to outside of 1 gallon Ziploc. This label will include participant ID, visit time-point, expiration date of kit, and field center

Tissue Biopsy Kits:

Tissue biopsy kits are site specific. The Tufts University field center will be collecting 4 extra muscle tissue biopsy samples that the other two sites will not be collecting.

1. Take out supplies needed for Tissue biopsy kit:
 - 1 - One-gallon Ziploc bag
 - 2 - Pint Ziploc bags
 - 1 - Tissue cassette (sample #24)
 - 1 - 4 oz Corning Snap-Seal container
 - 1 - 5mL vial (sample #02)
 - 12 (16 for Tufts kit) - 2mL Corning cryovials
 - Sample #'s 01, 03, 08, 21, 22, 23, 25-30 (PBRC and Wash. U)
 - Sample #'s 01, 03-08, 21, 22, 23, 25-30 (Tufts)
2. The Tissue biopsy kit has two components: 1) Muscle and 2) Adipose biopsy supplies
3. Use the special pen to write the kit ID directly on the cassette.
4. Affix appropriate labels to the cryovials (see step 1 for sample #'s)
5. Affix kit component label to the outside of each the 1 gallon Ziploc.
6. Fold P/P form in half and put form and transfer tubes into the 1 gallon Ziploc.
7. Put sealed 1 gallon Ziploc into the 2 gallon Ziploc with all the other visit supplies.
8. Affix Kit contents label to outside of 2 gallon Ziploc. This label will include participant ID, visit time-point, and field center. (Note: this kit will not have an expiration, date as there are no components included with an out-date.)

C. 3M, 6M and 18M Kit preparation

The supplies needed for these visits are site and visit specific, but do not differ between male and female.

Supplies needed prior to assembly:

- 3M, 6M, or 18M Label Set –next available participant ID needed at site
 - Forms - “3M, 6M, 18M visit” P/P Form (1)
 - Ziploc bags - pint (1) and quart (1)
 - Draw tubes - 10mL Serum (1), 10mL EDTA (1)
 - Cryovials 1.5mL skirted w/ color-coded caps - red-capped (4), purple-capped (4)
 - Kit label (includes site, visit, participant ID, and expiration date of kit)
1. Take out supplies needed for the kit you are preparing (see supplies list above).
 2. Affix appropriate labels to the draw tubes, cryos, and a form label to the P/P form.
 3. Put labeled draw tubes and cryos into the quart size Ziploc.
 4. Fold P/P form in half and put form, quart Ziploc (with tubes and cryos) into the quart Ziploc.
 5. Affix Kit contents label to outside of 1 quart Ziploc. This label will include participant ID, visit time-point, expiration date of kit, and field center

D. 17M, 23M and Unscheduled Visit Kit Preparation

The supplies needed for these visits are site and visit specific, but do not differ between male and female.

Supplies needed prior to assembly:

- 17M, 23M, or unscheduled visit Label Set –next available participant ID needed at site
 - Forms - “17M, 23M, or unscheduled visit” P/P Form (1)
 - Ziploc bags - quart (1)
 - Draw tubes - 10mL Serum (1)
 - 10mL Transfer tube (1)
 - Kit label (includes site, visit, participant ID, and expiration date of kit)
1. Take out supplies needed for the kit you are preparing (see supplies list above).
 2. Affix appropriate labels to the draw tube (#01), transfer tube (#02), and a form label to the P/P form.
 3. Put labeled draw tube and transfer tube into the quart size Ziploc.
 4. Fold P/P form and put form into the quart Ziploc with the tubes.
 5. Affix Kit contents label to outside of 1 gallon Ziploc. This label will include participant ID, visit time-point, expiration date of kit, and field center.

E. Inventory and Tracking of Kits

The following steps are to be completed by a CALERIE Core lab certified research laboratory technician.

The LCBP is responsible for tracking the expiration of kits being used at the sites. If expiration dates were not available at the time kits were added to the inventory they must be entered now.

- 1.) Open the CALERIE label-printing database (CALERIELabelPrinting.adp) located in folder [\\Med15\Shared\Groups\LCBP\Databases\CALERIE](#). The kit preparation and

label printing functions can also be opened from the Sample Management database main form.

- 2.) Select *Kit Preparation* from the main form. Select the *Site* and *Visit* of the kit prepared. Kits that have been prepared to date will appear in the table. Scroll down the bottom for the most recent kits prepared.

CALERIE - Add/Edit Kits Prepared

Add/Edit Kits Prepared

Site & Visit Selection

Site: Washington Univer

Visit: Baseline

Defaults for new kits

Date Prepared: 5/25/2008

Expiration Date:

Add Next Kit

Kit Label	Kit ID	For Female	Date Prepared	Expiration Date	Date Received	SiteID	VisitID
3056 00	3056	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3057 00	3057	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3058 00	3058	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3059 00	3059	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3060 00	3060	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3061 00	3061	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3062 00	3062	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3063 00	3063	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3064 00	3064	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3065 00	3065	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00

View Kits Prepared Close

Record: 1 of 65

- 3.) Click in the *Expiration Date* cell of the kit id(s) you wish to edit. Enter the correct expiration date for each id as appropriate. Once finished, click *Close*.

Kit Label	Kit ID	For Female	Date Prepared	Expiration Date	Date Received	SiteID	VisitID
3056 00	3056	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3057 00	3057	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3058 00	3058	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3059 00	3059	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3060 00	3060	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3061 00	3061	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3062 00	3062	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3063 00	3063	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3064 00	3064	<input checked="" type="checkbox"/>	4/28/2008	11/30/2008		3	00
3065 00	3065	<input checked="" type="checkbox"/>	4/28/2008			3	00

View Kits Prepared Close

Record: 65 of 65

Once a month, the expiration dates of kits prepared must be reviewed to confirm if expired kits have not been received by the CALERIE sites (i.e. available for use).

- 1) To check for expired kits, select the Kit Preparation form from the *CALERIE Label Printing-Main Form*.

CALERIE Label Printing - Main Form

CALERIE **CALERIE Label Printing**

Label Printing

☐ Vial Labels for Kits and Aliquots

☐ Box Labels

☐ Box Labels for Annual Visit Repository

Kit Preparation

☒ Kit Preparation

Exit

- 2.) The *Add/Edit Kits Prepared* form will appear. Select *View Kits Prepared* at the bottom of this screen.

F. Preparation of EDTA tubes with 10% Sodium Metabisulfite Additive

The following steps are to be completed by a CALERIE Core lab certified research laboratory technician.

Catecholamine concentrations will be measured in *arterialized venous blood*. This blood draw will be performed as part of the CALERIE Study **Baseline, Visit 7, Month 12, Visit 4, and Month 24, Visit 4** lab work. Tubes for this study will be the first of the series drawn. Blood will be injected via syringe into EDTA tubes containing a 10% sodium metabisulfite additive. The CALERIE Core Lab Research Technicians will prepare these tubes and ship as requested by the sites. As prepared, the EDTA-Sodium Metabisulfite tubes have an outdate period of 30 days.

Prepare a 10% solution of Sodium metabisulfite as follows:

1. Measure out 500mL of de-ionized water into a 1000mL beaker.
2. Use scale to measure out 50mg of powdered sodium metabisulfite (located on dry chemical shelf behind Mary Ellen's workbench).
3. Add 50mg of sodium metabisulfite to 500mL water.
4. Stir on stir plate with a small stir bar until powder dissolves into solution.
5. Label bottle with date prepared, contents and tech initials (Example: 10% Sodium metabisulfite prepared 3/26/07 by RHB). Please store prepared solution at room temperature in the basket labeled "CALERIE Hot Box Tube supplies" located on the second shelf above the Phlebotomy bench in T205.
6. Alert Rebekah that solution has been made so she can make labels to affix to draw tubes when they are prepared.

Note: This solution will expire after 30 days.

Inject EDTA tubes with 10% sodium metabisulfite as follows:

At the phlebotomy bench in T205, you'll find a basket labeled "CALERIE Hot Box Tube supplies." In this basket you will find:

- ✓ 1cc Tuberculin syringes
- ✓ 10% sodium metabisulfite solution
- ✓ 6mL EDTA tubes
- ✓ Labels for prepared draw tubes with expiration date (see Rebekah if none are available)

Additional supplies needed:

- ✓ P100 pipette and tips
- ✓ 100µL conical microcentrifuge tubes

* Size of microcentrifuge tubes and pipettes used will vary based on quantity of tubes being prepared at once. Because of the 30 day outdate on these tubes once prepared, plan to send about 10-12 tubes at a time to the sites or as requested.

1. Set up 6mL EDTA tubes to be filled in a rack

2. Aliquot 30µL of 10% sodium metabisulfite solution into a 100µL microcentrifuge tube.
3. Open a tuberculin syringe from wrapping.
4. Draw up 30µL (.03cc) sodium metabisulfite from microcentrifuge tube into syringe.
5. Inject syringe into rubber stopper of 6mL EDTA vacutainer tube. Vacutainer suction will draw the 30µL of solution from syringe into draw tube.
6. Repeat for the remaining 9 draw tubes. Same syringe can be used for all tubes prepared that day.
7. Dispose of syringe in sharps container.
8. Affix a sodium metabisulfite label to the prepared draw tubes (see Rebekah for labels if none are in the supply basket).

Once all tubes for the current month's supply have been made, dispose of prepared solution of sodium metabisulfite and remove label from glass bottle.

G. Packaging and Shipping of the CALERIE Kits and Supplies to the Field Centers

The following steps are to be completed by a CALERIE Core lab certified research laboratory technician.

Upon request from the sites, LCBR lab technicians will be sending kits and other supplies listed below to the CALRIE sites:

Shipped together from LCBR:

- ✓ Boric Acid
- ✓ 4mL Serum tubes (discard tubes)
- ✓ Transfer pipettes
- ✓ Shipping labels - IATA 650 Category B-Biological Substances, UN1845 dry ice, and "keep frozen."

Shipped separately:

- 6mL EDTA tubes with 10% sodium metabisulfite (prepared on pre-determined schedule)
- Thermosafe Insulated Shippers (6/case, sent directly from manufacturer to sites)

CALERIE Site Supply Shipping Addresses

Attn: Bridget Connor (blood/urine kits) Attn: Stacy Carling (Tissue kits)
Pennington Biomedical Research Center
 6400 Perkins Road
 Baton Rouge, LA 70808

Phone number: (225) 763-3047 Email: Elizabeth.Soroe@pbrc.edu
 OR Stacy.Carling@pbrc.edu

Attn: Stephanie Leon
Tufts University
USDA Human Nutrition Research Center on Aging
711 Washington St.
Boston, MA 02111
Phone: (617) 556-3143 Email: stephanie.leon@tufts.edu

Attn: Morgan Schram
Washington University School of Medicine
Division of Applied Physiology
660 S. Euclid Ave.
Campus Box 8113
St. Louis, MO 63110
Phone: (314) 747-3182 Email: mschram@im.wustl.edu

IV. PROCEDURES FOR RECEIVING BLOOD SAMPLES

The following procedures are to be performed by Research Laboratory Technicians whom have received certification in CALERIE study Sample Receipt Training.

A. Shipping Schedule and Notification/Tracking

The CALERIE samples will arrive in monthly shipments (the first week of each month) from the three study field centers (Tufts University, Washington University, and Pennington Biomedical Research Center).

Prior to shipping, each site will send notification via fax with a list of expected sample IDs and any FedEx tracking numbers. Retrieve all faxed shipping notification forms and check FedEx website for tracking information. Note any missing/late packages and contact the site immediately.

B. Receipt of the Shipment Containers

Things to Do Before Samples Arrive:

Be sure to have enough sample boxes made and ready for the incoming samples. See Box maps for grid size, tape color, labels.

Clear off the CALERIE Shelves in T154C -80°C REVCO number 2.

Once Samples arrive:

- Record all shipments by site and sender on the sample receipt log book (located on a clear clip-board on the shelf above the work bench)
- Visually inspect condition of shipping containers, labeling.

- Open all boxes and check condition of samples.
- Remove and organize paperwork by visit type.
- Carefully remove sample boxes, confirm against paperwork, and place in temporary storage location.
- Record any sample condition problems or discrepancies.
- Record received date on all forms and indicate if samples were received frozen

After everything is verified, put the samples in the sample receipt -80°C freezer in a location dedicated for temporary CALERIE samples. If there is not enough space on the CALERIE shelves, there is additional space available on the other shelf just below.

C. Data Entry of Shipping, Phlebotomy, and Processing Forms

1. Separate the forms by visit and site.
2. When applicable, staple all of the loose pages for the same CALERIE ID and same visit together so that the Phlebotomy form is on top and the Processing form is on bottom. If for any reason any other forms are sent for the same ID, staple them after (behind) the processing form. (Sometimes sites will send extra forms, such as site specific forms.)
3. Record the received date on the processing form and circle Y or N to indicate if they were frozen upon arrival.
4. Put the stapled pages together so that the participant IDs match in order with the shipping form. Also put the sites together in numerical order, starting with site 1, Pennington.
5. Open the CALERIE Sample Management Database. The CALERIE database can be found at the following location:

L:\Groups\LCBR\Databases\CALERIE\
CALERIESampleMgmt_MED27_20080205.adp (or the most current version of the database saved in this location)

CALERIE Sample Management - Main Form

Sample Processing

- ☐ Enter Shipping Forms
- ☐ Enter Collection Forms
- ☐ Scan Received Vials
- ☐ Scan LCBR Aliquots

Participant Info

- ☐ Enter Participant Info
- ☐ View Ppt Kit Info
- ☐ Reporting to CC
- ☐ Sample Repository
- ☐ QA/Reliability

Kits & Labels

- ☐ Label Printing
- ☐ Kit Preparation

Assay Processing

- ☐ FAHC Assay Work
- ☐ LCBR Assay Work
- ☐ Assay Results

- If this is the baseline visit for a new CALERIE ID, the first step will be entering the CALERIE ID into the database. Under the section heading “**Participant Info**”, click on the option “Enter New Participant”. Enter the requested information for each new CALERIE ID received.

CALERIE - Participant Info

Participant Info

Edit Mode

- ☒ Add new participants
- ☐ Edit existing participants

Locate CALERIE ID

Locate Kit

CALERIE ID

DOB

Gender

RcptID

Kits received for this participant

Kit Label	Date Received	ReposID	Visit

Record: of 1

Scanning in Shipping Forms:

- Next, on the main screen, Under the section heading “*Sample Receipt*”, click on the “Shipping Forms” option to open the data entry screen.

CALERIE - Enter Shipping Forms / Kits Received

CALERIE *Enter Shipping Forms / Kits Received*

Select Date Received
(or enter a new one)
3/4/2009

Scan Kit Labels here

Kit Label	CALERIE ID	Date Rcvd	Frozen	Site	Visit	Kit ID	RcptID
3031 12	03-0039	3/4/2009	✓	Washington Univ	12-Month	3031	730
3093 00	03-0133	3/4/2009	✓	Washington Univ	Baseline	3093	731
3032 12	03-0047	3/4/2009	✓	Washington Univ	12-Month	3032	732
3019 12	03-0049	3/4/2009	✓	Washington Univ	12-Month	3019	733
3094 00	03-0138	3/4/2009	✓	Washington Univ	Baseline	3094	734
3003 18	03-0007	3/4/2009	✓	Washington Univ	18-Month	3003	735
3040 03	03-0123	3/4/2009	✓	Washington Univ	3-Month	3040	736
3043 03	03-0109	3/4/2009	✓	Washington Univ	3-Month	3043	737
3044 03	03-0105	3/4/2009	✓	Washington Univ	3-Month	3044	738
3045 06	03-0079	3/4/2009	✓	Washington Univ	6-Month	3045	739
3046 06	03-0081	3/4/2009	✓	Washington Univ	6-Month	3046	740
3047 06	03-0076	3/4/2009	✓	Washington Univ	6-Month	3047	741
3048 06	03-0097	3/4/2009	✓	Washington Univ	6-Month	3048	742

☐ Shipping Form Verification Report

Record: 1 of 17

- Begin scanning in the barcodes from the shipping forms.
- Each time a new shipping label is scanned a CALERIE ID must be selected from the drop down list.
- Click on Frozen if samples arrived in an acceptable frozen condition.
- Print Verification Report and then close out of this screen.

Entering Collection Forms:

- Once all of the IDs have been scanned into the database, move onto the collection form entry.
- Again at the main screen, select “Collection Forms” under the section “Sample Receipt.”
- Choose date received and visit type for the paperwork to be entered.
- A list will appear of all shipping labels that have been scanned in matching the date and visit type.
- The first kit ID in the list of kit’s received will automatically be highlighted and display the collection forms to be entered for that kit id.

CALERIE - Collection Forms

Collection Forms

Kit Receipt Batch Selection

Visit Type:

Date Received:

Kits Received

KitLabel	PptID
3031 12	03-0039
3093 00	03-0133
3032 12	03-0047
3019 12	03-0049
3094 00	03-0138

Kit Label: **CALERIE ID:**

Catech P/P **Fasting Phleb** **Fasting Processing** **OGTT P/P** **Urine** **Day 2 Sex Hormone** **Tubes Collected/Rcvd**

Blood Collection Date **Kit ID**

Phleb Tech ID **Visit ID**

Needle Gauge

IV Location
☒ Dorsal hand vein ☐ Other

Supine Position?
☒ Yes ☐ No

#01 Collection Start Time **Blood Collected?**
☒ Tube #01

#02 Collection Start Time ☒ Tube #02

Proc Tech ID **Comments**

Centrifuge Start Time

☐ View Verification Reports

- The Catecholamine draw phlebotomy form will be the front tab that appears. Fill in the information requested from the Catecholamine P/P form for that participant. Next click on the last tab "Tubes Collected/Received" and fill in requested information under "Collection Tubes" and "Shipping Tubes".

CALERIE - Collection Forms

Collection Forms

Kit Receipt Batch Selection

Visit Type: BL, 12M, 24M

Date Received: 3/4/2009

Kits Received

KitLabel	PptID
3031 12	03-0039
3093 00	03-0133
3032 12	03-0047
3019 12	03-0049
3094 00	03-0138

Kit Label: 3031 12 CALERIE ID: 03-0039

Catech P/P Fasting Phleb Fasting Processing OGTT P/P Urine Day 2 Sex Hormone Tubes Collected/Rcvd

Collection Tubes

Vial #	Filled?	Volume
01	Yes	
02	Yes	
03	Yes	
04	Yes	
05	Yes	
06	Yes	
07	Yes	
08	Yes	
09	Yes	
10	Yes	2.5
11	Yes	2.5
12	Yes	
13	Yes	
14	Yes	
15	Yes	
16	No	
17	Yes	

Shipping Tubes

Vial #	Partial	Hemolyzed	Sent	Volume	Comment
10	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	2.5	
11	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	2.5	
20	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	2.5	
21	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	2.5	
22	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	1.0	
23	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	1.0	
24	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	1.0	
25	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	2.0	
26	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	5.0	
27	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	5.0	
28	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	5.0	
29	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	0.5	
30	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	0.5	
31	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	1.0	
32	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	1.0	
33	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1.0	

☐ View Verification Reports

Record: 14 of 5

7. Enter this data in the same fashion for the Fasting, OGTT, and Urine.

Questions that may arise:

1. Note all partial or hemolyzed tube on P/P forms, and check the appropriate boxes under the "Tubes Collected/Received" tab.
2. If there is a discrepancy between what is received and what is indicated on the forms, email the site to resolve (cc: Rebekah).

10. Repeat steps 1-7 for all visit types received in shipment. Tissue form entry is completed in the same fashion. Enter all information requested from the biopsy worksheets. Be sure to correctly record tissue cryovials, sample vials and IHC cassettes sent as they appear on the biopsy worksheet.

CALERIE - Collection Forms

Collection Forms

Kit Receipt Batch Selection

Visit Type: Tissue - BL,12M,24M

Date Received: 3/4/2009

Kits Received

KitLabel	PptID
3003 82	03-0042
3030 80	03-0138
3004 82	03-0049

Tissue Collection Data

Kit Label: 3003 82

CALERIE ID: 03-0042

Kit ID: 3003

Visit ID: 82

Date of Biopsies: 30-Jan-09

Physician: Dr. Villareal

Assistant: MLU/AF

Prep by: MLU

Comments: No Fat, did not get at baseline.

☐ IHC cassette arrived at room temperature

Samples Collected

Vial #	Sent	Weight (mg)	Comment
01	<input checked="" type="checkbox"/>	57.6	
02	<input checked="" type="checkbox"/>	20.0	
03	<input checked="" type="checkbox"/>	30.0	
04	<input type="checkbox"/>	0.0	
05	<input type="checkbox"/>	0.0	
06	<input type="checkbox"/>	0.0	
07	<input type="checkbox"/>	0.0	
08	<input type="checkbox"/>	0.0	
21	<input type="checkbox"/>	0.0	
22	<input type="checkbox"/>	0.0	
23	<input type="checkbox"/>	0.0	
24	<input type="checkbox"/>	0.0	
25	<input type="checkbox"/>	0.0	
26	<input type="checkbox"/>	0.0	
27	<input type="checkbox"/>	0.0	
28	<input type="checkbox"/>	0.0	
29	<input type="checkbox"/>	0.0	
30	<input type="checkbox"/>	0.0	

11. Once all paperwork is entered for a specific visit type click “View Verification Reports” at the bottom of the “Collection Forms” screen.
12. Print all verification files and verify form entry. **ALL OF THE PAPERWORK MUST BE VERIFIED BEFORE SCANNING!**
13. If any data entry errors are found, open the appropriate screen of the database and make the edit needed. Circle the incorrect data on the verification report, make a note on the verification report: *edit made to database*, write correct data, initial and date the verification report.

D. Scanning the Samples

Blood and Urine Samples Scanning

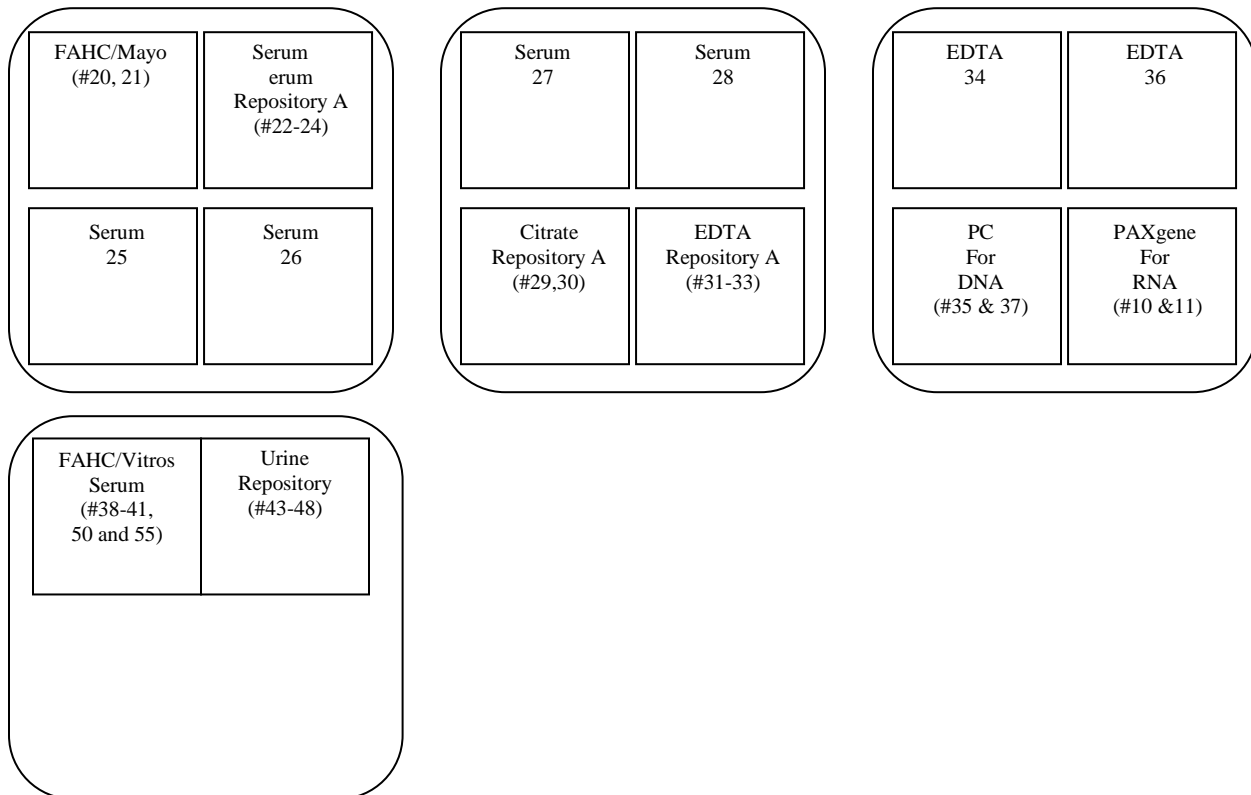
1. After all paperwork is entered and verified for all visit types, the received samples are ready to be scanned.

- Set up baseline visit repository boxes and tubs of dry ice as needed to ensure that the samples remain frozen through the scanning process.

Set up the dry ice bins in the following manner:

Use Box Maps listed in the Appendix B for orientation of cryovials and tubes within boxes.

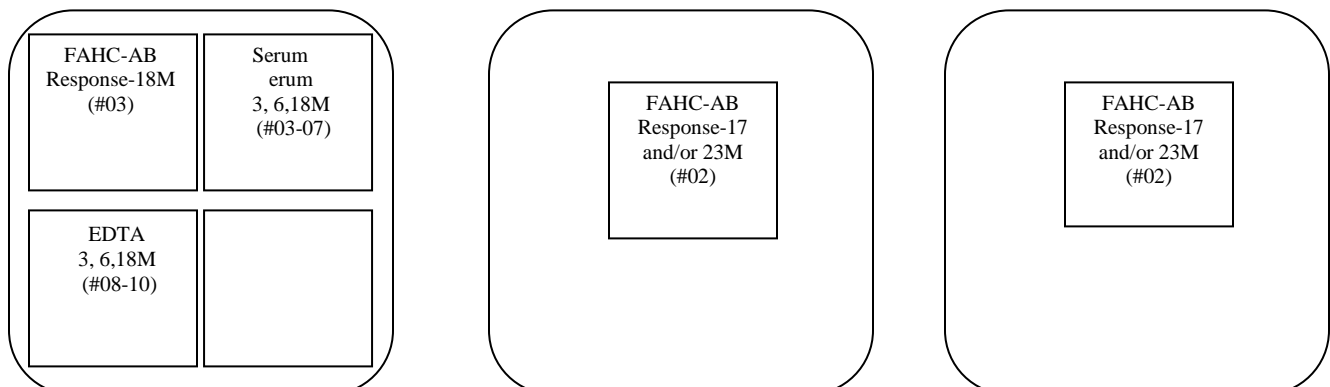
Annual Visit boxes (Baseline, 12M and 24M):



3, 6 and 18 Month Visit boxes

17 and/ or 23 Month Visit box

Unscheduled Visit



3. From the main menu, under the section “Sample Receipt”, choose “Received Vial Scanning”. Select the date and visit type. The first kit id scanned in from the forms will automatically be highlighted.

RcptID	KitLabel	PptID	ReposID
61	2003 00	02-0022	13
62	2004 00	02-0024	14
63	2005 00	02-0003	15

4. A list will appear of all the vials that should have been received, according to the P/P forms. The left-hand column lists the samples sent and the right-hand column shows the samples received. Verify that vials shown as “sent” were included in the shipment. Any discrepancies should be verified against the P/P forms.

CALERIE - Received Vial Scanning

Calerie **Received Vial Scanning**

Kit Receipt Batch Selection

Visit Type: BL,12M,24M
Date Received: 3/4/2009

Scan Vial Here:
Vial To Scan: 3031 12 10

Kits to Scan

KitLabel	PptID	ReposID
3031 12	03-0039	200
3093 00	03-0133	201
3032 12	03-0047	202
3019 12	03-0049	203
3094 00	03-0138	204

Vials to Scan for Selected Kit

Vial Label	Vial #	Sent	Rcvd	DNE	Sample Type	Purpose
3031 12 10	10	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Paxgene	RNA
3031 12 11	11	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Paxgene	RNA
3031 12 20	20	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	EDTA Nor Epi	Catecholamine
3031 12 21	21	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	EDTA Nor Epi	Catecholamine
3031 12 22	22	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Serum	Repository A
3031 12 23	23	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Serum	Repository A
3031 12 24	24	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Serum	Repository A
3031 12 25	25	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Serum	LCBR To Aliquot
3031 12 26	26	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Serum	LCBR To Aliquot
3031 12 27	27	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Serum	LCBR To Aliquot
3031 12 28	28	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Serum	LCBR To Aliquot
3031 12 29	29	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Citrate	Repository
3031 12 30	30	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Citrate	Repository
3031 12 31	31	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	EDTA	Repository A
3031 12 32	32	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	EDTA	Repository A
3031 12 33	33	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	EDTA	Repository A
3031 12 34	34	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	EDTA	LCBR To Aliquot

☐ Scan Summary Report
☐ All Dates Received
☒ All Visit Types

☐ Mark Vial as DNE ☐ Edit Vial Details

Record: 1 of 5

- Start at the top of the list and scan the received vials in the order shown placing the samples into the appropriate repository box. Annual Visit Box assignments will be designated as follows:

Box	ID extensions that go into it
FAHC/Mayo	EDTA tube _20 and 21
Serum Repository A	Serum cryo _22 thru _24
Serum 25	Serum tubes _25
Serum 26	Serum tubes _26
Serum 27	Serum tubes _27
Serum 28	Serum tubes _28
Citrate Repository A	Citrate cryos _29 and _30
EDTA Repository A	EDTA cryos _31 thru _33
EDTA 34	EDTA tube _34
EDTA 36	EDTA tube _36
PC for DNA	Red Cells tubes _35 and _37
PAXgene for RNA	PAXgene _10 and _11
OGTT Serum	Serum tube _38 thru _41
Urine Repository	Urine tubes _43 thru _48

- When finished scanning in the first kit ID, verify that all the samples recorded as "received" also appear as "sent." If a sample was neither sent nor received you MUST mark vials as DNE. Select the appropriate vial by clicking to the left and then click "Mark Vial as DNE".
-

8. As the boxes fill up, rubber-band them and place them in a separate CALERIE section in the -80°C REVCO until the end of the day when scanning is done.
9. Repeat scanning process (steps 1-7) for all kits of each visit type. All sample sets must be scanned in the same order as they are listed on the shipping forms. Empty participant boxes should be returned to each site for future use.

Blood and urine sample box assignments for other visits are as follows:

Box	ID extensions that go into it
Serum Repository-3M, 6M, and/or 18M	Serum cryo _03 thru _06
EDTA Repository-3M, 6M, and/or 18M	EDTA cryo _07 thru _10
FAHC AB Response-18M	Serum cryo _03
FAHC AB Response-17 and/or 23M	Serum tube _02
FAHC/Centaur-Unscheduled (Women only)	Serum tube _02

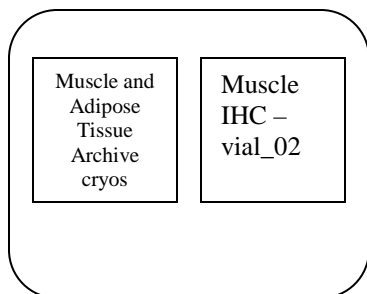
When all of the participant blood and urine samples have been scanned, move on to scanning in the annual visit tissue samples.

Tissue Sample Scanning

1. After all tissue biopsy worksheets are entered and verified, the received tissue samples are ready to be scanned.
2. Set up tissue repository boxes and tubs of dry ice as needed to ensure that the samples remain frozen through the scanning process. Adipose tissue cassettes (#24) are stored at room temperature.

Set up the dry ice bins in the following manner:

Use Box Maps listed in Appendix B for orientation of cryovials and vials within boxes.



3. As with the blood and urine sample scanning, from the main menu, choose “Received Vial Scanning” and select the Date and Tissue. The first kit scanned in from the shipping forms will automatically appear highlighted on the “Kits to Scan” list.

4. Under “Vials to Scan for Selected Kit” a list will appear of all the vials that should have been received, according to the biopsy worksheets. The left-hand column lists the samples sent, and the right-hand column shows the samples received. Verify that vials shown as “sent” were included in the shipment. Any discrepancies should be verified against the tissue biopsy worksheets. Again, if a sample was neither sent nor received you **MUST** mark vials as DNE.
5. Start at the top of the list and scan the received vials in the order shown placing the samples into the appropriate repository box.

See tissue box maps in Appendix section *B.1 SAMPLE RECEIPT BOXES*:

Box	ID extentions that go into it
Muscle IHC	Vial_02
Adipose IHC (cassettes)	Cassette_24
Tissue RNA and Archive	Cryos_01, _03 thru _08, _21 thru _23, _25 thru _30

This should conclude the scanning for the samples.

Clinic Acknowledgements:

Contact the site immediately if:

- ✓ The expected shipment did not arrive (include any FedEx tracking or correspondence regarding shipment).
- ✓ Any forms or other paperwork is missing (so they can fax it).
- ✓ There are any discrepancies or questions regarding labeling, cryo order, cap color, tube condition, etc., **especially if there are any questions regarding potential participant**

mix-ups. No question is too small to ask; correcting minor mistakes now will save us from major headaches later!

- ✓ Any other issues that arise involving any specific site where an immediate response is necessary.
- ✓ Copy the Project Manager on any discrepancies and their resolutions.

E. Aliquotting of Baseline, 12M, or 24M Serum and EDTA tubes

Of the 30 tubes or cryos that are received for each participant's baseline, 12M, or 24M visit, 10 tubes/cryos need to be aliquotted further. See appendix C. for the table of the cryo/tube #'s that need to be aliquotted as well as the order in which the aliquots should be made. Samples received from the CALERIE sites at other visits (3M, 6M, 18M, etc) do not require any aliquotting at LCBR. Each participant is aliquotted independently of other participant samples to ensure only 1 participant's specimen aliquots are aliquotted at a time.

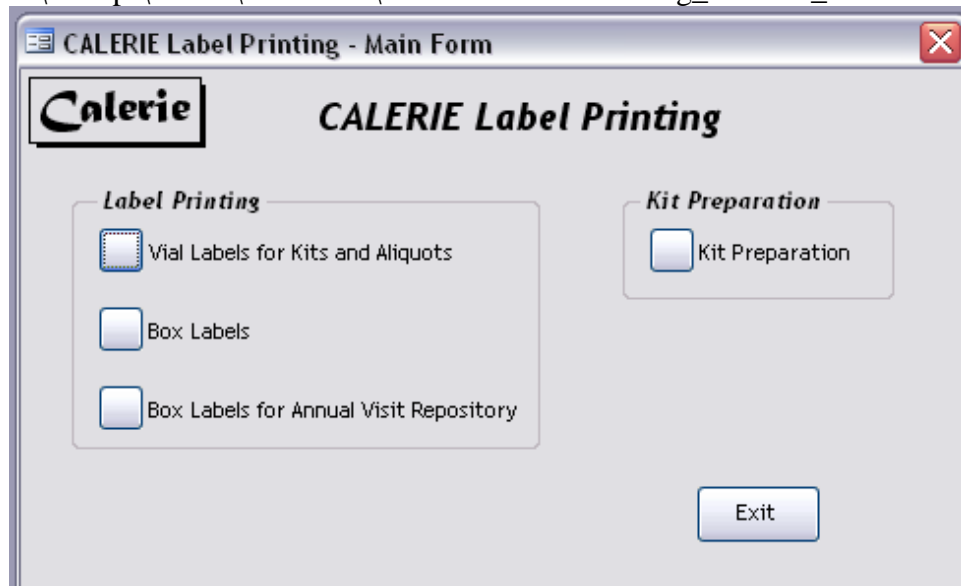
Aliquot Labels

Prepare for aliquotting samples by printing all vial labels needed. Each new participant will need an annual visit repository box. Each kit ID will need a set of LCBR aliquot labels.

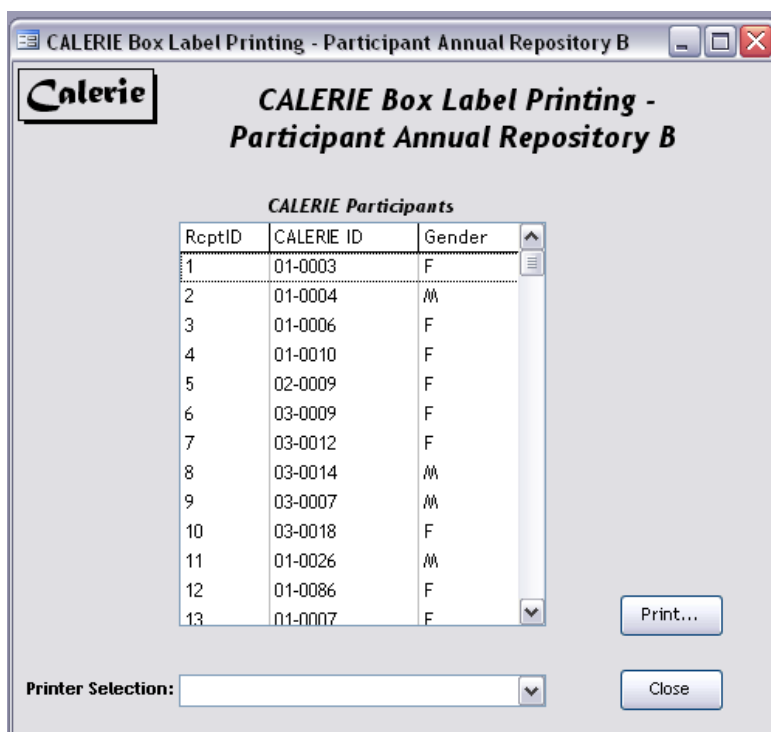
Printing Box labels for Annual Visit Repository:

1. Open the *CALERIE Label Printing* database found in:

L:\Groups\LCBR\Databases\CALERIELabelPrinting_MED27_20080110.adp



2. At the main screen select *Box Labels for Annual Visit Repository*.



3. Scroll down the list of CALERIE participants and select the ID that you want to print.
4. Click on the printer selection drop down menu and select the printer you are using from the list.
5. Be sure the printer is set up the printer with 4-across labels. And select *Print*.

Printing Box labels for other boxes:

1. Again go to the CALERIE label printing main screen and select *Box Labels*.
2. Select the appropriate visit and box type from the screens on the left.
3. On the right of the screen, adjust the *Starting Tower*, *Starting Box*, and *Number of Boxes* to reflect the next consecutive set of labels needed.

CALERIE Box Label Printing

Visit Type Selection

- 1 BL, 12M, 24M
- 2 3M, 6M, 18M
- 3 17M, 23M, UN
- 4 Tissue - BL, 12M, 24M
- 5 Off-Cycle - BL, 12M, 24M
- 9 Reliability

Box Range

Starting Tower: 1

Starting Box: 1

Number of Boxes: 1

Box Type Selection

BoxType	VialsPerPpt	PptsPerBox	BoxesPerTower
Paxgene RNA	2	21	8
Serum Repository A	3	33	11
EDTA Repository A	3	33	11
Citrate Repository	2	49	11
EDTA Packed Cells	2	24	7
Urine Repository	6	7	7
Serum OGTT - Kristal	4	25	11
FAHC - Catecholamines	2	24	
FAHC - Imulite	1	49	
FAHC - Vitros	7	7	

Boxes Per Tower:

Ppts Per Box:

Starting Box ID: 1

Starting ReposID:

Ending ReposID:

Printer Selection:

Print... Close

4. Select your printer from the drop-down list at the bottom.
5. Be sure the printer is loaded with 4-across labels and click *Print*.
6. Do steps 1-5 for any other visit type and box type combination that is needed.

Printing Aliquot Labels:

1. Go to the main *CALERIE Label Printing* screen and select *Vials Labels for Kits and Aliquots*.

CALERIE Vial Label Printing

Visit Selection

- 0 Baseline
- 3 3-Month
- 6 6-Month
- 12 12-Month
- 17 17-Month
- 18 18-Month
- 23 23-Month
- 24 24-Month
- 70 Baseline Off-Cycle
- 72 12-Month Off-Cycl
- 74 24-Month Off-Cycl
- 80 Baseline Tissue
- 82 12-Month Tissue

Label Selection

☒ Print all labels shown below

LabelNum	VialNum	Descr1	Descr2
62	50	Serum	Lipid Profile
63	51	Serum	Cortisol
64	52	Serum	Progest /LH,FSH,SHBG,Tst
65	53	Serum	Repository B
66	54	Serum	TSH,T3
67	55	Serum	Glucose
68	56	Serum	DHEAS
69	57	Serum	Reliability~Gluc,DHEAS,T3
70	58	Serum	Repository B
71	59	Serum	Repository B
72	60	Serum	Repository B
73	61	Serum	Repository B
74	62	Serum	Repository B
76	65	Serum	CRP,ICAM,IL6
77	66	Serum	GH,IGF-1,BP1,BP3
78	67	Serum	Adiponectin,TGF-b1,PDGFAB
79	68	Serum	CTX,PINP
80	69	Serum	Reliability-IGF1,CRP,ICAM

Label Group

☐ All labels

☐ Kit labels

☒ LCBR aliquot labels

Kit ID Range

Start: 1057

End: 1057

Printer Selection:

Print... Close

2. Select the appropriate visit type under *Visit Selection* and *Site*.
3. Select *LCBR Aliquot Labels* from the *Label Group Selection* on the top right.
4. Type in the *Kit ID Range* needed.
5. If you need to print an entire label set, be sure that the check for *print all labels shown below* is selected. Otherwise click on the specific labels you'd like to print (Ctrl click for more than one selection and a time)
6. Select the appropriate printer from the *Printer Selection* menu at the bottom of the screen.
7. Be sure label printer is loaded with 5-across labels and click *Print*.

Serum Transfer Tube (Tubes 25-28) Aliquotting:

1. Label aliquot vials ahead of time to minimize the amount of time the samples remain at room temperature.
2. Label the appropriate tubes (4mL false-bottom, 12x75mm, and 0.5 mL or 1.5mL cryovial) according to the Aliquotting Guide (**See Appendix C**).
3. Thaw serum tubes #25, 26, 27 and 28 in a 37°C water bath. Total thaw time should be approximately 5-7 minutes. Invert tubes at least once during thaw.
4. Once tubes have completely thawed, pool all four serum tubes into one 50mL conical tube.
5. Invert pooled serum tube 15 times and aliquot according to aliquotting guide. Pooled serum will remain at room temperature during the aliquotting process. Serum aliquotting will take less than 10 minutes to complete for each participant.
6. Put aliquots upright into -80°C freezer immediately after prepared.

OGTT Tube Aliquotting:

1. Label the Tubes 38-41 aliquots as indicated in the Aliquotting Guide.
2. Thaw all 4 OGTT tubes together in water bath. Invert tubes at least once during thaw.
3. Mix thawed tubes by inverting each tube 15 times prior to aliquotting.
4. Aliquot according to Aliquotting Guide. OGTT serum tubes will remain at room temperature during the aliquotting process. OGTT serum aliquotting will take less than 5 minutes to complete for each participant.

- Put aliquots upright into -80°C freezer immediately after prepared.

EDTA Aliquotting:

- Label tube #34 and #36 aliquot vials ahead of time to minimize the amount of time the samples remain at room temperature.
- Thaw tube #34 and 36 in a 37°C water bath. Invert tubes at least once during thawing.
- Mix tube #34 by inverting 15 times, then aliquot into 0.5 mL cryovials according to the aliquotting guide. Put aliquotted cryovials immediately into -80°C freezer.
- Mix tube #36 by inverting 15 times, then aliquot into 0.5 mL cryovials according to aliquotting guide. EDTA tubes will remain at room temperature during the aliquotting process. EDTA aliquotting will take less than 10 minutes to complete for each participant.

F. Aliquotting of Serum High Controls, Low Controls and Control Sets:

Serum Sets are composed of 20 - 1.0mL aliquots of normal human serum. Each aliquot is a different person and each serum set is identical. After all participant samples have been aliquotted and stored in -80C, serum high and low controls and/or serum sets aliquots can be made. Remember that thawed serum samples and controls should not be left out at room temperature for more than 15 minutes.

Serum Sets will be created and sent along with regular samples to FAHC once every 3 months. Serum high and low controls will be created and sent over to FAHC with each sample set that is sent over the FAHC (which means that at minimum these will be sent monthly).

For the purposes of CALERIE assay quality control, we need to create a serum set to analyze for each of the CALERIE FAHC tests requested using serum. So essentially we will be sending over a set of 20 for each of the 7 tests/vial #'s listed below (n=140 tubes):

Table 1. Serum Controls Tube and Volume Requirements

Vial #	Test	Tube Type	Instrument	Aliquot Volume
50	Lipid Profile	Tall	Vitros	600
51	Cortisol	12 x 75	Centaur	300
52	LH, FSH, SHBG, Testosterone	12 x 75	Centaur	500
54	TSH.T3	12 x 75	Centaur	500
55	Glucose	tall	Vitros	200
56	DHEAs	12 x 75	Immulite	500
75	C-Peptide	12 x 75	Mayo	500

We will send these sets over to FAHC for testing every 3 months.

Serum Control Set Label Design:



The first 2 digits indicate that these are serum set ids. The next 2 digits are the serum set id (01-20). The last 2 digits are the vial # (50, 51, 52, 54, 55, 56, or 75). These labels will be the same each time we send over a serum set.

Serum High and Low Control Label Design:



The first 4 digits indicate that these are either the high or low serum control (777= low, 888=high). The next 2 digits are the vial # (50, 51, 52, 54, 55, 56, or 75). These labels will be the same each time we send over serum controls to FAHC.

Refer to **Section III A** for instructions of printing serum set and serum high and lo control labels

Quarterly Serum Set Aliquotting Procedure:

- 1) Label tubes needed for aliquotting. Refer to **Table 1** for tube sizes needed specific to each vial #.
- 2) Take out one box of serum sets from freezer. The towers of serum sets kept at CRF are located in Xanadu #1. 3.1mL of serum will be required for testing of each of the 20 samples in the control set. Each cryovials contains ~1.0mL serum thus a minimum of 4 sets of 20 will be required every three months.

- 3) To be sure to have enough volume to create the 7 assay tubes per sample #, thaw 4 sets of 20 cryovials in a 37C water bath.
- 4) Set up racks for aliquotting. Decide what set-up works best for you, but 1 suggestion is: Line up the 20 - 4mL Sarstedt tubes for #50/ Lipid profile testing chronologically (1 thru 20) from left to right in a rack. In the next row down line up the 20 - 12x 75 tubes for #51/Cortisol testing chronologically from left to right in the same rack. Continue in the same fashion for the other 6 vial #'s/tests (See rack map below).

Table 2. Serum Set Aliquoting Rack Map

	Serum Set Id #																			
Vial #50	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
Vial #51	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
Vial #52	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
Vial #54	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
Vial #55	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
Vial #56	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
Vial #75	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Once tubes are labeled and organized for aliquotting and cryovials have thawed, take out the first set of 20 cryovials from the freezer box. Put freezer box with remaining sets in the refrigerator while you aliquot the first serum set.

- 5) Set pipette to 600uL for aliquotting of the Lipid panel test/Vial #50 serum set. Be sure to mix each serum set cryovials by inverting 15 x times before removing volume for aliquot.
- 6) Aliquot the first serum set into the appropriate vial #50 cryovials. Remember to change tips between serum set ids and vortex each cryovial gently before aliquoting each sample.
- 7) Once you have completed aliquotting the Lipid profile/Vial #50 serum set, put the set in the to FAHC Vitros box with the other CALERIE sample being sent over the FAHC.
- 8) Repeat steps 6-8 for each test/vial# referring to Table 1 for aliquot volumes as you go. Once you have depleted a serum set, retrieve another set from the box in the refrigerator and continue.

- 9) You should have approximately 700uL remaining in the last serum set once you have created all the FAHC serum sets. Draw a black line on the cryo caps for this last set and put back into the -80C freezer. (This will indicate that these samples have received 1 thaw prior to the next use)

Monthly Serum High and Low Control Aliquotting Procedure:

- 1) Label tubes needed for aliquotting. Refer to **Table 1** for tube sizes needed specific to each vial #.
- 2) Remove one vial of each level control from the freezer. Allow vials to thaw completely in 37C water bath.
- 3) Refer to Table 1 for aliquot volumes and prepare aliquots accordingly. Mix control vials by inverting 15 x before pipetting aliquots. Be sure to change pipette tips between aliquotting of high and low control vials.
- 4) Put aliquotted samples into appropriate FAHC testing boxes and freeze at -80C immediately.

Scanning LCBR Aliquots:

1. All aliquots, except for serum high/low controls and serum control sets, must be scanned into the database. Samples are to remain frozen on dry ice during this process.
2. From the main page of the CALERIE database under “Sample Receipt” select “Aliquot Scanning”.
3. Select the date received of the sample you are about to scan.
4. A list of Kit IDs that were received that day will appear. Select the first kit ID.
5. A list of aliquots to be scanned will appear on the bottom of the screen. Scan the aliquots prepared. The order of scanning here is not important. Return samples immediately to -80C freezer once scanning is complete.

CALERIE - LCBR Aliquot Scanning

Calerie **LCBR Aliquot Scanning**

Kit Receipt Batch Selection

Visit Type: BL,12M,24M

Date Received: 3/4/2009

Scan Aliquots Made Here

NB: The aliquots for the current kit may be scanned in any order.

Kits to Scan

KitLabel	PptID	ReposID
3031 12	03-0039	200
3093 00	03-0133	201
3032 12	03-0047	202
3019 12	03-0049	203
3094 00	03-0138	204

Aliquots for Selected Kit

Vial Label	Vial #	Scanned	DNE	Scan Date	Sample Type	Purpose
3031 12 08	08	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 13:10	Serum 30min	C-Peptide
3031 12 09	09	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 13:10	Serum 60min	C-Peptide
3031 12 12	12	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 13:10	Serum 90min	C-Peptide
3031 12 13	13	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 13:10	Serum 120min	C-Peptide
3031 12 14	14	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 12:58	Serum 30min	Insulin
3031 12 15	15	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 12:58	Serum 60min	Insulin
3031 12 16	16	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 12:58	Serum 90min	Insulin
3031 12 17	17	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 12:59	Serum 120min	Insulin
3031 12 18	18	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 12:58	Serum OGTT	Repository
3031 12 19	19	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 12:58	Serum OGTT	Repository
3031 12 38	38	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 13:10	Serum 30min	OGTT
3031 12 39	39	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 13:10	Serum 60min	OGTT
3031 12 40	40	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 13:10	Serum 90min	OGTT
3031 12 41	41	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 13:10	Serum 120min	OGTT
3031 12 50	50	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 14:16	Serum	Lipid Profile
3031 12 51	51	<input checked="" type="checkbox"/>	<input type="checkbox"/>	3/16/09 14:16	Serum	Cortisol

☐ Scan Summary Report
☐ All Dates Received
☒ All Visit Types

☐ Mark Aliquot as 'Not Made'

☐ Edit Vial Details

Close

Record: 1 of 5

- It is very important to mark aliquot as not made if appropriate at this screen. Click to the left of the vial not created and then click on the “mark aliquot as *not made*” at the bottom of the screen.
- Go back to the main screen and under “Repository” select “Repository Viewing”. **It is critical to report volumes that differ from the expected or default aliquot volumes at this point.** Type in appropriate kit labels that need to be edited and click “retrieve records”. Make any edits to volume as needed. Complete for other kit IDs as needed.

Calerie

Repository Viewing

Specify viewing criteria

CALERIE ID

Kit

VisitID/Vial#

Box Type

Status

Retrieve Records

Vial Label	CALERIE ID	ReposID	Tower	Box	Col	Row	Volume	Thaws	Status	Comment	Sample Type	Purpose
1004 00 10	01-0026	10	1	1	4	1	2.50	1	1		Paxgene	RNA
1004 00 11	01-0026	10	1	1	4	2	2.50	1	1		Paxgene	RNA
1004 00 18	01-0026	10	1	1	4	7	0.80	5	5		Serum OGTT	Reserved-Kristal
1004 00 19	01-0026	10	1	1	4	8	0.80	5	5		Serum OGTT	Reserved-Kristal
1004 00 22	01-0026	10	1	1	4	1	1.00	1	1		Serum	Repository A
1004 00 23	01-0026	10	1	1	4	2	1.00	1	1		Serum	Repository A
1004 00 24	01-0026	10	1	1	4	3	1.00	1	1		Serum	Repository A
1004 00 29	01-0026	10	1	1	2	9	0.50	1	1		Citrate	Repository
1004 00 30	01-0026	10	1	1	2	10	0.50	1	1		Citrate	Repository
1004 00 31	01-0026	10	1	1	4	1	1.00	1	1		EDTA	Repository A
1004 00 32	01-0026	10	1	1	4	2	1.00	1	1		EDTA	Repository A
1004 00 33	01-0026	10	1	1	4	3	1.00	1	1		EDTA	Repository A
1004 00 35	01-0026	10	1	1	3	5	5.00	1	1		EDTA Packed	DNA Extraction
1004 00 37	01-0026	10	1	1	3	6	5.00	1	1		EDTA Packed	DNA Extraction
1004 00 43	01-0026	10	1	2	3	1	9.00	1	1		Urine	Repository
1004 00 44	01-0026	10	1	2	3	2	9.00	1	1		Urine	Repository
1004 00 45	01-0026	10	1	2	3	3	9.00	1	1		Urine	Repository
1004 00 46	01-0026	10	1	2	3	4	9.00	1	1		Urine & Acid	Repository
1004 00 47	01-0026	10	1	2	3	5	9.00	1	1		Urine & Acid	Repository

G. FAHC Laboratory Testing Batches

- Under “Assay Processing” on the CALERIE main page, select “Assay Batch Management.”
- Highlight the first Batch Type desired (*example: Catecholamine*), and a list will appear containing all vials that are still un-batched.

CALERIE - Assay Batch Management

Calerie Assay Batch Management

Assay Location: ☒ FAHC ☐ LCBR

Batch Type: 1 Catecholamine, 7 Lipid Panel, **2 Cortisol**, 6 LH,FSH,FTES, 11 TSH,T3, 13 Glucose, 16 DHEA-Sulfate, 3 C-Peptide, 14 Amenorrhea, 4 DHEA-Sulfate,Insulin (o), 9 Glucose,Insulin (obs)

Unbatched Vials: 19 vials

Label	PptID	Gender	DateRcvd
1001 12 51	01-0004	M	7/8/2008
1061 00 51	01-0129	F	7/8/2008
1064 00 51	01-0127	M	7/8/2008
1065 00 51	01-0140	M	7/8/2008
3056 00 51	03-0070	F	7/10/2008
3044 00 51	03-0038	M	7/10/2008
3057 00 51	03-0072	F	7/10/2008
3058 00 51	03-0076	F	7/10/2008
3059 00 51	03-0082	F	7/10/2008
3045 00 51	03-0003	M	7/10/2008

Locate BatchID:

Create Batch

Delete Batch...

Batches of Selected Type

BatchID	BatchDate
230	4/6/2009
221	3/16/2009
211	2/24/2009
203	2/12/2009
195	1/29/2009
187	12/8/2008
178	11/7/2008
172	10/13/2008
163	9/16/2008
145	7/8/2008
138	8/14/2008
120	5/10/2008

Vials in Selected Batch: 11 vials

Label	PptID	Gender	DateRcvd
1094 00 51	01-0230	M	3/3/2009
1104 00 51	01-0232	F	3/3/2009
1105 00 51	01-0234	F	3/3/2009
1106 00 51	01-0237	F	3/3/2009
1044 12 51	01-0100	F	3/3/2009
1045 12 51	01-0103	F	3/3/2009
3031 12 51	03-0039	F	3/4/2009
3093 00 51	03-0133	F	3/4/2009
3032 12 51	03-0047	F	3/4/2009
3019 12 51	03-0049	M	3/4/2009
3004 00 51	03-0138	F	3/4/2009

Generate Assay Request List

View Results Verif Report

Close

3. Select each desired vial individually to add to a batch, or select "Create Batch" to automatically add all vials.
4. A new Batch ID will appear with no date listed next to it. Highlight this batch ID and select "Generate Assay Request List."
5. A date will now be listed next to the batch ID, and an Excel spreadsheet will be created in the 'Files to FAHC' folder in the CALERIE database. (L:\ drive location: L:\Groups\LCBR\Databases\CALERIE\Files to FAHC) Each new batch request from this date will create a new worksheet in the workbook of this Excel file.
6. Repeat steps 2 through 4 for all desired Batch Types, then close and return to the 'Files to FAHC' folder.
7. Create a new folder with the date the samples are sent to FAHC, and drag the Excel file into this folder.
8. Manually add any controls as necessary to the individual worksheets. Serum set controls, high and low serum controls are not scanned into the database.
9. Alert Project Manager that the batches are prepared and the electronic file is saved in the CALERIE data folder. Project Manager will prepare an electronic file to send to

FAHC/Judy Schwenn prior to samples being brought to the chemistry lab with the courier. (see example of test request report on p.34)

10. Print out hardcopies of all worksheets in this file to be included in the sample boxes along with the vials. Double-check that all vials are present in the samples boxes.
11. Store sample boxes in -80°C until courier is available for transporting samples to FAHC.
12. Courier will pick up samples for delivery to FAHC on Tuesdays and Thursdays. Courier will take CALERIE samples from a CALERIE technician and store in a cooler with dry ice. If courier does not have dry ice, we provide. Courier will immediately drive to FAHC where he will hand deliver samples to the FAHC hospital chemistry lab sample receipt technicians.

Example of Electronic Test Request Report:

FAHC Chemistry Lab Assay Request - LCBR

Study: CALERIE	Date of Request: 5/19/2008
Location Code: GCAL	
Bill to MRN: 9614207	
Ordering Doctor: 05 04 35	
Contact: Elaine Cornell - LCBR	Number of Samples: 0
Phone: 656-8963	
Email: elaine.cornell@uvm.edu	

<i>Sample #</i>	<i>Sample ID</i>	<i>Sample Type</i>	<i>Test Requested</i>
	10580051	Serum	CORT
	20410051	Serum	CORT
	7777 51	Serum	CORT
	8888 51	Serum	CORT
	10580075	Serum	CPEP
	20410075	Serum	CPEP
	7777 75	Serum	CPEP
	8888 75	Serum	CPEP
	10580056	Serum	DHES
	20410056	Serum	DHES
	7777 56	Serum	DHES
	8888 56	Serum	DHES
	20410052	Serum	LH,FSH,FTES
	7777 52	Serum	LH,FSH,FTES
	8888 52	Serum	LH,FSH,FTES
	10580050	Serum	LPR
	20410050	Serum	LPR
	7777 50	Serum	LPR
	8888 50	Serum	LPR
	10580054	Serum	TSH,T3
	20410054	Serum	TSH,T3
	7777 54	Serum	TSH,T3
	8888 54	Serum	TSH,T3
	10580055	Serum	SGL
	20410055	Serum	SGL
	7777 55	Serum	SGL
	8888 55	Serum	SGL

End of Processing Procedures

- ✓ Put all P/P forms in CALERIE ID order.
- ✓ Put all of the verification forms in order by site.
- ✓ Put all shipping forms in order by site.
- ✓ Put all P/P forms into appropriate drawers of designated filing cabinet. Clearly label all new hanging file folders.
- ✓ Put all verification forms into there appropriate folder separated by site.
- ✓ Put all of the shipping forms into appropriate folder separated by site and filed by date received.
- ✓ Next remove all completed cryo boxes out of the freezer and put them onto a cart with tubs loaded with dry ice. Update the freezer log book and proceed in putting boxes into there appropriate towers. **Do not allow samples to thaw during this process.**
- ✓ Repository boxes are to be brought out to the Flynn Ave. Repository facility in a cooler with dry ice.

Housekeeping/Supplies and Mailer Return

- ✓ Replace any shipping boxes that are damaged beyond safe and effective use (torn, leaking etc.).
- ✓ Return the empty sample boxes in the returning shipper.
- ✓ Check with Rebekah to see if any supplies have been ordered by the sites.
- ✓ Return boxes via FedEx Express saver (if no supplies requested); if supplies were requested, use FedEx 2-Day Service.
- ✓ File the supply request form in the appropriate binder and affix the FedEx receipt on back for easy tracking.
- ✓ If supplies are low notify Nicole or Rebekah for restocking.
- ✓ Prepare sample boxes for next month's samples. (*one set of boxes*)

V. OVERVIEW OF REPOSITORY DESIGN AND TRACKING

The main CALERIE protocol calls for samples of blood, urine, and tissue to be procured for repository storage in the following quantities:

Sample type	Visits at baseline, 12 months and 24 months	Visits at 3 months, 6 months, and 18 months
Serum	10 ml	4 ml
Serum (OGTT)	4 ml	
Plasma (EDTA)	9 ml	4 ml
Plasma (citrate)	1 ml	
RNA	from 2 PAXgene tubes	
DNA	from packed cells from 2 EDTA tubes	
Urine (no additive)	18 ml	
Urine (with boric acid)	18 ml	
Skeletal muscle	~45 vials	
Adipose tissue	~30 vials	

Some of the aliquoting of samples will be performed at the field centers; the remainder will occur upon arrival at our laboratory. Regardless of location, personnel entrusted with this responsibility will have been appropriately trained and demonstrated competence in the application of the aliquoting procedure. Most of the aliquots for storage will measure either 0.5 ml or 1.0 ml in volume.

The implementation phase includes bringing the repository database online and procuring the physical resources, i.e., freezers, alarm systems, backup power, and physical space required to accommodate the quantity of samples generated.

Implementation of the CALERIE repository

The three -80°C ultra-cold chest freezers required for storage of cryovials containing blood and urine will be Revco/Thermo brand Ultima II-9 freezers (model ULT2090-9-D33), which are 20 cubic foot chest units with dual-stage compressors and a voltage (surge and sag) regulator. Each unit has the capacity for monitoring temperature failure, power failure, compressor failure and fuse/battery failure. Each freezer also has a data port for remote alarm connection. Additional storage supplies to be purchased will include freezer racks and boxes.

Skeletal muscle and adipose tissue samples will be stored in one to two Thermo CryoPlus2 liquid nitrogen (LN2) storage units (model 7402), each of which has a 24-inch tank capable of containing 200 liters of LN2. The evaporation rate is 5 liters per day with a static holding time of 40 days. Each unit has an auto-filling system and is capable of monitoring temperature, LN2 levels, and valve malfunctions. Each storage unit also has a data port for remote alarm connection. Additional supplies will include racks, boxes, and liquid nitrogen tanks.

Freezers and liquid nitrogen storage units comprising the CALERIE biosample repository will be installed in accordance the manufacturer's instructions and will reside in a new 14,000 square foot University of Vermont facility located approximately five miles from the main LCBR facility at Flynn Ave. This building features a temperature controlled environment (65°F) and full generator back-up capacity (automatic) that will run for 24 hours using 344 gallons of fuel; the generator is maintained and tested on a monthly basis. Access to the building is limited, and it is equipped with an intrusion alarm.

For alarm monitoring, an autodialer (Sensaphone model 1108) will be connected to all freezers and storage units to monitor unit temperature, power failure, and ambient temperature. This instrument is capable of calling a list of phone numbers repeatedly until the alarm is acknowledged by certified LCBR staff. The first number dialed is that of a beeper that is monitored by on-call staff 24 hours per day, seven days per week. The Sensaphone also has a call-in capacity for remote monitoring of conditions by certified staff. Fiber-optic cable is being run to the facility, and once this is completed, we will shift to a web-based primary system with phone dialing backup. We have a certified freezer repair service on call 24 hours per day, seven days per week.

Operation and Maintenance of the CALERIE repository

Operation and maintenance of the repository will be carried out in accordance with the principles outlined in the document titled "NHLBI Biological Samples: Recommendations for Standardized Storage Protocols" Vials to be stored in the repository will be labeled with barcodes to facilitate accurate, efficient data entry, and each vial will be scanned upon entry into the repository. Database fields for each sample will include the participant identifier, the visit number, the aliquot number, sample type, sample volume, and location.

We will generate quarterly reports for the Coordinating Center, the Steering Committee, and other appropriate committees concerning the status of the repository; these reports will include a detailed inventory of the numbers and types of samples received and stored, and any issues related to the physical equipment will also be documented. The data contained in the reports will be sortable, which will permit detailed analysis with respect to fields such as sample ID, CALERIE ID, visit number, or volumes stored, among others. Additional reports will be generated as needed or as requested by the Coordinating Center or by committees such as the Steering Committee.

Monitoring of the repository will follow the following schedule:

Frequency	Type of monitoring
Daily	Temperature and status of freezers and LN2 storage units Visual inspection
Monthly	Alarm testing Generator testing
Quarterly	Preventive maintenance
Semiannually	HVAC system maintenance

Each of the types of monitoring described above will be documented, as will each occasion on which a sample is placed in or removed from a freezer or storage unit. Protocols for dealing with power outages or freezer/storage unit malfunctions will be posted in the facility, as will names and phone numbers of key personnel to be contacted if such events are noted by security or maintenance personnel. A contract with a maintenance service available 24 hours a day, 7 days a week will be maintained. Access to the repository facility is appropriately limited, and it is equipped with intrusion alarms. Emergency power for the facility is provided by generators fueled sufficiently to provide 24 hours of backup electricity.

VI. **DESCRIPTION OF LABORATORY ASSAY METHODS** *(to be completed)*

CALERIE Core Lab Assay List

LCBR Assays	FAHC Assays
IL-1a (Linco cytokine panel)	Glucose* (fasting and OGTT)
IL-8 (Linco cytokine panel)	C-Peptide (fasting and OGTT)
IL-6 (Elisa)	Norepinephrine
TNFa (Linco cytokine panel)	DHEA(S)*
ICAM-1 (Elisa)*	Cortisol
Insulin (Fasting and OGTT)	TSH
CRP (BNII)*	T3*
MCP-1 (Linco panel B)	LH (men)
TGF-B1 (Elisa)	FSH (men)
Leptin (Linco Panel B)	LH, FSH, Estradiol (women) (estimate 30 total)
Adiponectin (total)	SHBG
Angiotensin II	Free Testosterone
GH	Total Testosterone
IGF-1 (Elisa)*	
IGF-BP1 (Elisa)	AB Response pneumoc vaccines (BL, 17M, 18M, 24M)
IGF-BP3 (Elisa)	AB response tetanus vaccines (BL, 17M, 18M, 24M)
PDGFAB (Elisa)	AB response hepatitis B vaccines (BL, 17M, 18M, 23M, 24M)
Serum CTx (plus 6 M visit)	
PINP (plus 6 M visit)	
Extraction Kits for Paxgene tubes (2 per person + 5%)	
Extraction Kits for DNA (+5%)	

* = Reliability Study Assays

FAHC Assay Method Summaries

Hardcopy protocols provide by FAHC Chemistry laboratory are located in the CALERIE SOP binder in the CALERIE Core Lab Sample Receipt laboratory.

Lipid Profile (total cholesterol, HDL-cholesterol, triglyceride, calculated LDL)

These assays are run at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, an affiliate of the University of Vermont. The Ortho Vitros Clinical Chemistry System 950IRC instrument (Johnson & Johnson Clinical Diagnostics, Rochester, NY), which uses thin film technology, is used to quantitatively measure lipid levels via a colorimetric reaction.

Cholesterol is measured using a colorimetric reflectance spectrophotometric method. Normal ranges for adults are Desireable : < 200 mg/dL; Borderline: 200 – 239 mg/dL; High: \geq 240 mg/dL. The reportable range for this assay is 50-325mg/dl. The expected CV of this assay is <2%.

Direct HDL Cholesterol is assayed by colorimetric reflectance spectrophotometry after samples are treated with phosphitungstic acid/magnesium chloride to precipitate HDLs and non-HDLs. Normal ranges for adults are Highly Desireable: > 60 mg/dL; Desireable: 35-60 mg/dL; High Risk: <40 mg/dL. The reportable range for this assay is 5.0-110.0 mg/dL. The expected CV of this assay is approximately 7%.

LDL Cholesterol is calculated: $\text{Total Cholesterol} - \{\text{HDL} + (\text{Triglycerides}/5)\}$. Normal ranges for adults are Desireable: < 130 mg/dL; Borderline: 130-159 mg/dL; High Risk: \geq 160 mg/dL.

Triglycerides are measured by colorimetric reflectance spectrophotometry. Normal ranges for adults are Normal:<150 mg/dL; Borderline High: 150-199 mg/dL; High; Very High: \geq 150mg/dL . The reportable range for this assay is 10.0-525.0 mg/dL. The expected CV of this assay is <2%.

Glucose: Glucose is measured using a colorimetric reflectance spectrophotometric method on the Vitros 950IRC instrument at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, an affiliate of the University of Vermont. The normal range for serum glucose in healthy fasting adults is 74-106 mg/dL. The reportable range for the serum glucose assay is 20.0–625.0 mg/dl with an expected CV of approximately 1%.

Estradiol: Estradiol-6 is measured using a sequential chemiluminescent immunoassay on the Advia Centaur instrument (Bayer Diagnostics Corp., East Walpole, MA) at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, an affiliate of the University of Vermont. The analytical measuring range for this assay is 10 to 1000 pg/mL. The inter-assay and intra assay % CVs range from 4.5-8.1 and 4.0-12.1 respectively.

Testosterone (Total): Total Testosterone is measured using a competitive direct chemiluminescent immunoassay on the Advia Centaur instrument (Siemens Healthcare Diagnostics, Tarrytown, NY) at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, an affiliate of the University of Vermont. The analytical measuring range for this assay is 10 to 1500 ng/dL. Normal range for males is 5.0 to 24.0 ng/dl and females is 0.1 to 1.5ng/dl.

Testosterone (Free): Free Testosterone is calculated: $\text{Concentration Testosterone} = \text{Free Testosterone} + \text{Albumin Bound Testosterone} + \text{SHBG-Bound Testosterone}$. The normal reference range for males is 5.0 to 24.0 ng/dl and females is 0.1 to 1.5 ng/dl.

Sex Hormone Binding Globulin (SHBG): SHBG is measured using a solid-phase, two-site chemiluminescent immunoassay on the DPC Immulite 2500 instrument (Siemens Healthcare Diagnostics, Tarrytown, NY) at the Clinical Chemistry Laboratory at Fletcher Allen Healthcare, an affiliate of the University of Vermont. The reference range for this assay is Males: 13-71 nmol/L and Females (non-pregnant): 18-114 nmol/L. This assay has an intra-assay %CV range of 2.4% to 7.0%.

Follicular Stimulating Hormone (FSH): FSH is measured using a two-site chemiluminometric sandwich immunoassay on the Advia Centaur instrument (Siemens Healthcare Diagnostics, Tarrytown, NY) at the Clinical Chemistry Laboratory at Fletcher Allen Health Care. The reportable range for this assay is 0.3 to 150.0 mIU/mL. The inter-assay and intra assay %CV ranges are 0.3 to 2.7% and 2.0 to 2.9% respectively.

Norepinephrine: Norepinephrine is measured using High-Pressure Liquid Chromatography by Laboratory Medicine and Pathology at the Mayo Clinic in Rochester, MN. The reference ranges for this assay are Supine: 70- 750 pg/mL and Standing: 200- 1,700 pg/mL.

C-Peptide: C-Peptide is measured by a two-site sandwich immunoassay on the ACS 180 automated immuno-analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY) using a direct chemiluminescent detection-technology. Analysis is performed by Laboratory Medicine and Pathology at the Mayo Clinic in Rochester, MN. The reference ranges for this assay is 297 - 1419 pmol/L.

Cortisol: Cortisol is measured using a competitive direct chemiluminescent immunoassay on the Advia Centaur instrument (Siemens Healthcare Diagnostics, Tarrytown, NY) at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, an affiliate of the University of Vermont. The analytical measuring range for this assay is 0.5- 70 ug/dL. The inter-assay and intra assay %CV ranges are 1.86 to 5.45% and 2.89 to 3.82% respectively.

Thyroid Stimulating Hormone (TSH): TSH is measured using a two-site chemiluminometric sandwich immunoassay on the Advia Centaur instrument (Siemens Healthcare Diagnostics, Tarrytown, NY) at the Clinical Chemistry Laboratory at Fletcher Allen Health Care. The reportable range for this assay is 0.02 to 125.0 uIU/mL. The inter-assay and intra assay %CV ranges are 1.18 to 4.47% and 2.67 to 4.35% respectively.

Triiodothyronine (T3): T3 is measured using a competitive immunoassay on the Advia Centaur instrument (Siemens Healthcare Diagnostics, Tarrytown, NY) at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, an affiliate of the University of Vermont. The analytical measuring range for this assay is 20 to 800 ng/dL. The inter-assay and intra assay %CV ranges are 0.99 to 1.33% and 1.45 to 3.18% respectively.

DHEA-Sulfate (DHEA-S): DHEA-S is measured using a one cycle solid-phase, competitive chemiluminescent enzyme immunoassay on the DPC Immulite 2500 instrument (Siemens Healthcare Diagnostics, Tarrytown, NY) at the Clinical Chemistry Laboratory at Fletcher Allen Healthcare, an affiliate of the University of Vermont. The analytical range for this assay is 15-1000 ug/dL. This assay has an intra-assay %CV range of 4.9% to 9.8%.

LCBR Assay Method Summaries *(pending completion)*

Hardcopy protocols of currently defined assay are located in the SOP file cabinet at the LCBR.

C-reactive protein is measured using the BNII nephelometer from Dade Behring utilizing a particle enhanced immunonephelometric assay. Polystyrene particles are coated with monoclonal antibodies to CRP, which, in the presence of antigen (CRP) agglutinate to cause an increase in the intensity of scattered light. The increase in scattered light is proportional to the amount of CRP in the sample. Suitable specimens for this assay are serum, heparin, or EDTA anticoagulated plasma. The assay range is 0.16 – 1100 ug/mL. Expected values for CRP in normal, healthy individuals are ≤ 3 mg/L. Intra-assay CVs range from 2.3 – 4.4% and inter-assay CVs range from 2.1 – 5.7%.

Insulin: Insulin is measured by an ultra sensitive sandwich type enzyme immunoassay (Alpco Diagnostics; Salem, New Hampshire). The detectable range for the assay is 0 – 20uIU/mL. In healthy adults, mean Insulin levels range from 5-25 uIU/mL. Inter-assay and Intra-assay CVs range from 5.6-8.1% and 2.2-3.9% respectively.

Insulin-like growth factor-1 (IGF-1): IGF-1 is measured using a high sensitivity ELISA (Human IGF-1 Immunoassay, R&D Systems, Minneapolis, MN). The assay range is 0.94-6.0 ng/ml. The normal range of IGF-1 in serum and plasma is 40–258 ng/ml. Intra-assay and inter-assay CVs range from 3.5-4.3% and 7.5-8.3%, respectively.

Tumor necrosis factor- α (TNF- α): TNF- α is measured by Luminex technology multiplex ELISA using the Human Serum Adipokine Panel B LINCOplex Kit (Linco Research, Inc.; St. Charles, MO): The minimum detectable level of TNF- α is 0.14 pg/mL and the assay range is 0.64 – 10,000 pg/ml. Intra-assay CVs range from 1.4-7.9% and inter-assays CVs are generally < 21%.

Leptin: Leptin is measured by Luminex technology multiplex ELISA using the Human Serum Adipokine Panel B LINCOplex Kit (Linco Research, Inc.; St. Charles, MO). The assay range for this analyte is 16 – 250,000 pg/ml. Inter-assays CVs are generally < 21%.

Monocyte Chemotactic Protein-1 (MCP-1): MCP-1 is measured by Luminex technology multiplex ELISA using the Human Serum Adipokine Panel B LINCOplex Kit (Linco Research, Inc.; St. Charles, MO). The assay range is 3.2 – 50,000 pg/ml. Intra-assay CVs range from 1.4-7.9% and inter-assays CVs are generally < 21%.

LCBR Assay	Category by CALERIE	Manufacturer	Catalog#	Sample type	Vol (uL)
IL1a, IL-8	Inflammation	Linco cytokine panel	HCYTO-60K	EDTA	200
Tnfa,MCP-1, Leptin	Inflammation (Leptin is Endocrine response)	Linco Panel B	HADK2-61K-B	EDTA	200
IL-6	Inflammation	R&D chemiluminescent	Q6000B	Serum	230
ICAM-1	Inflammation	R&D Elisa or Bender Med Systems	<i>pending</i>	Serum	25
CRP	Inflammation	BNII Nephelometer	OQ1Y21, OQ1Y13	Serum	200
Adiponectin	Endocrine Response	R&D Elisa	DRP300	Serum	10
TGF-B1	Transforming Growth Factor	R&D Elisa	DB100	Serum	120
Angiotensin II	Endocrine Response	Alpco RIA	01-RK-A22	Serum	500
Insulin	Glucose Tolerance/Insulin	Alpco Ultrasensitive EIA	<i>80-INSHUU-E01</i>	Serum	50
GH	Growth Hormones	DSL	<i>active? Ultra-sensitive?</i>	Serum	100
IGF-1	Growth Hormones/Endocrine	DSL	DSL-10-2800	Serum	20
IGF-BP1	Growth Hormones	DSL	DSL-10-7800	Serum	200
IGF-BP3	Growth Hormones/Endocrine	DSL	DSL-10-6600	Serum	10
PDGFAB	Growth Hormones/Endocrine	R&D Elisa	DHD00B	Serum	10
Serum CTX (also at 6M)	Collagen Turnover & Fibrosis	Orion Diagnostica /UniQRIA	06097	Serum	100
PI NP (also at 6M)	Collagen Turnover & Fibrosis	Orion Diagnostica /UniQRIA	06096	Serum	120

Clinical Chemistry Assays	Category by CALERIE	Method	Analyzer	Sample type	Vol (uL)
Glucose (fasting and OGTT)	Glucose Tolerance/Insulin	Colorimetric Reflectance Spectrophotometry	Vitros	Serum	200
C-Peptide	Glucose Tolerance/Insulin	Chemiluminometric immunoassay		Serum	300
Catecholamines	Endocrine Response	High-Pressure Liquid Chromatography		EDTA	2000
DHEA-s	Endocrine Response	Chemiluminescence Immunoassay	Immulite	Serum	400
Cortisol	Endocrine Response	Chemiluminescence Immunoassay	Centaur	Serum	300
TSH and T3	Endocrine Response	Chemiluminescence Immunoassay	Centaur	Serum	500
Progesterone (women only)	Sex Hormones	Chemiluminescence Immunoassay	Centaur	Serum	500
LH (men & amenorrhea)	Sex Hormones	Chemiluminescence Immunoassay	Centaur	Serum	500
FSH (men & amenorrhea)	Sex Hormones	Chemiluminescence Immunoassay	Centaur	Serum	500
Estradiol (amenorrhea)	Sex Hormones	Chemiluminescence Immunoassay	Centaur	Serum	400
SHBG (men only)	Sex Hormones	Immulite Solid Phase 2-site Chemi Immunoassay	Immulite	Serum	500
Free & Total Testosterone (men only)	Sex Hormones	Equilibrium Dialysis, Chemi Immunoassay	Centaur	Serum	500
Lipid Profile	Serum Lipids and Lipoproteins	Colorimetric Reflectance Spectrophotometry	Vitros	Serum	600
<i>Pneumococcal Vaccine Response</i>	AB response	<i>method pending</i>		Serum	
<i>Tetanus Toxoid IgG Antibody</i>	AB response	<i>method pending</i>		Serum	
<i>Hep A antibody</i>	AB response	<i>method pending</i>		Serum	

VII. PROCEDURES FOR ONGOING QUALITY CONTROL IN THE LABORATORY

A. Laboratory testing and analysis control

- Function checks to verify stability and validity of samples:
 - We believe that sample drift over time in a freezer to be virtually non-existent for most classes of biomarkers including general chemistry assays, and many micro- and macro-nutrients, and all proteins examined so far. We have published our experience with a large variety of protein markers: Lewis MR, et al. Longitudinal stability of coagulation, fibrinolysis, and inflammation factors in stored plasma samples. [*Thromb Haemost.* 2001;86:1495-500.](#)
- Control checks:
 - We test control preparations with each run, track control results using Levey-Jennings charts, and apply Westgard Multirule QC to ensure quality assay performance.
 - Our control materials come in lyophilized and frozen forms; as we have published (reference noted above), the stability of these materials over time is excellent.
 - Our procedures ensure that lyophilized controls are reconstituted properly and that frozen controls are thawed properly to ensure optimal performance; for example, making certain that temperatures for control materials for thawing do not exceed lab limits.
- Documentation, including tracking of events:
 - Performance of our control preparations is monitored using Levey-Jennings charts, and Westgard Multirule QC procedures are used to determine whether an analytical test is performing acceptably.
 - All QC data are documented, reviewed in real time, kept on file, and periodically audited. Any corrective action is documented in the appropriate QC files and raw data files.

B. Control of measuring and testing equipment

The laboratory is equipped with smaller equipment such as pH meters, centrifuges, analytical balances, etc. Available major equipment includes:

- Stago STA-R Automated Coagulation analyzer for coagulation and immunoturbidimetric assays
- Dade Behring BNII nephelometer
- BioTek Elx808 Ultra Microplate Reader for fluorescence and absorbance ELISA and Kinetic assays
- Molecular Devices Spectra Max 250 for fluorescence and absorbance ELISA and Kinetic assays
- Bio-Rad BioPlex Protein Array System 100-240V for fluorescent bead-based Luminex technology to simultaneously analyze up to 100 targets in a single microplate well
- Dynex Technologies MLX Microtiter Plate Luminometer for luminescence technology
- Cobra gamma counter for RIA
- MJ Research Programmable thermocyclers (4) for PCR
- ABI Prism 7900 DNA Sequence Detector for TaqMan-based SNP analysis

Certain assays are performed in the Clinical Laboratory of the Department of Pathology at Fletcher Allen Health Care (FAHC) using the following instrumentation:

- Tosoh 2.2 Plus hemoglobin A1C analyzer utilizing automated ion-exchange chromatography
- Ortho Clinical Diagnostics Vitros 950 and 250 chemistry analyzers
- Bayer Advia ACS:Centaur automated chemiluminescence analyzer
- Beckman Image used for microalbumin analysis
- Diagnostic Products Immulite for insulin measurement

- Calibration is scheduled and documented per our standard procedures based on manufacturer's specifications.
- The LCBR and the FAHC laboratory maintain service contracts to ensure appropriate maintenance of testing instruments and to assure availability of rapid response should issues requiring immediate service attention arise.

C. Preventive maintenance

- Our procedures call for performance of preventive maintenance tasks in accordance with recommendations from instrument manufacturers.
- As noted above, the LCBR and the FAHC laboratory maintain service contracts to ensure appropriate maintenance of testing instruments and to assure availability of rapid response should issues requiring immediate service attention arise.

D. Data validation

- Bar-coding of samples and electronic data transfer from instruments to databases are used whenever possible to avoid opportunities for human error.
- When human involvement in data entry or data transfer is unavoidable, we use a data entry and verification step to minimize the risk of transcription errors. Additional measures taken to ensure data integrity are:
 - Spot checks of data
 - Final reconciling of reported data vs. raw data
- Databases are developed using SQL, Visual Basic, and Microsoft Access to facilitate storage and retrieval of data for our various studies.

E. Nonconformity

- As noted above, for assay QC, Westgard multi-rule control procedures are used to determine whether an analytical test is performing acceptably.
- If non-conforming results are identified, the following steps are taken (Tetrault 2001:154-5):
 - The nature and severity of the problem are assessed:
 - Are there random non-conforming values, or is there evidence of systematic error (bias)?
 - What is the magnitude of the deviation, and for how long (i.e., over how many runs) has the deviation been present?
 - QC data are examined and guide the troubleshooting process:
 - If the non-conforming values appear to reflect random error, factors affecting precision (e.g., sample and reagent factors, external factors) are explored.
 - If there is suspicion of systematic deviation, factors influencing accuracy (e.g., stability of calibration) are assessed.
 - Potential drift and/or reagent lot change affects are monitored by assaying a control set of 20 normal donors over the length of the study.

F. Corrective action

- Corrective action follows the results of the process outlined in item 5. For example if the technologist responsible for the assay, in consultation with supervisory staff, identifies the cause or causes of the deviation, we then go on to identify steps to be taken to avoid similar scenarios in the future and to document the problem identification and resolution information. If additional training is found to be desirable, such training is provided and documented as well.

G. Quality documentation and records control

Documents such as sampling procedures, calibration procedures, analytical and test procedures, data collection and reporting are developed and modified based on individual study requirements. When appropriate, all critical documents are kept in locked, secured locations.

- Documentation of all QA and QC data pertaining to performance of assays in a given study are retained indefinitely (i.e., throughout the length of the study and beyond).
- Documents pertaining to ongoing work are maintained on-site in the LCBR.
- Changes are made only with approval of supervisory staff and are documented appropriately (i.e., time and date of change, name of person authorizing change).
- Changes to procedures are communicated to affected personnel immediately by the project manager and to the entire laboratory through our regular laboratory meeting and/or via e-mail.
- When LCBR staff are not regularly scheduled to be present, access to LCBR facilities is via card access only with documentation of entry based on card identification.

Additional issues for consideration

H. Longitudinal stability of assays

In multi-year studies, it is critical to be certain that assay performance remains stable over long periods of time. To help in this endeavor, we have adopted several tactics in the LCBR.

- As is our practice, in both multiplex and individual ELISA assays, care will be taken to minimize reagent lot changes over this project period.
 - Whenever possible, control materials are purchased in advance in quantities such that they will be available for the duration of the study.
 - Whenever possible, reagents are purchased in lots large enough to be used throughout a study.
 - A serum set of approximately 20 individuals is assayed at the start of the study for all baseline measurements and then reassayed periodically throughout the study and when there is any major reagent or instrument change in order to monitor potential assay drift.
- Lyophilized calibrators are also employed whenever possible to maximize assay stability.
- When appropriate, resampling of a selected subset of the original cohort is performed.

VIII. LABORATORY PERFORMANCE AND QC REPORTS FOR THE QC COMMITTEE

A. Monthly Vermont Core Lab QC Reports

The following reports will be submitted monthly to the CALERIE Coordinating Center.

MONTHLY Summary Report

Lab/Reading Center: VERMONT

Date: September 2007

Site: PBRC

Status at the End of this Month:	Timepoints					
	BL	6M	12M	18M	24M	Total
A. Total # of <u>subjects</u> with <u>new</u> samples/records received in the lab:	5	0	0	0	0	5
B. Total # of <u>subjects</u> whose samples/records have <u>completed</u> processing:	5	0	0	0	0	5
C. Total # of <u>subjects</u> whose samples/records are awaiting completion:	0	0	0	0	0	0
D. Total # of <u>subjects</u> whose samples/records have errors, problems, or queries, preventing processing from being completed:	0	0	0	0	0	0

Site: Tufts University

Status at the End of this Month:	Timepoints					
	BL	6M	12M	18M	24M	Total
A. Total # of <u>subjects</u> with <u>new</u> samples/records received in the lab:	5	0	0	0	0	5
B. Total # of <u>subjects</u> whose samples/records have <u>completed</u> processing:	5	0	0	0	0	5
C. Total # of <u>subjects</u> whose samples/records are awaiting completion:	0	0	0	0	0	0
D. Total # of <u>subjects</u> whose samples/records have errors, problems, or queries, preventing processing from being completed:	0	0	0	0	0	0

Site: Washington University

Status at the End of this Month:	Timepoints					
	BL	6M	12M	18M	24M	Total
A. Total # of <u>subjects</u> with <u>new</u> samples/records received in the lab:	4	0	0	0	0	4
B. Total # of <u>subjects</u> whose samples/records have <u>completed</u> processing:	4	0	0	0	0	4
C. Total # of <u>subjects</u> whose samples/records are awaiting completion:	0	0	0	0	0	0
D: Total # of <u>subjects</u> whose samples/records have errors, problems, or queries, preventing processing from being completed:	0	0	0	0	0	0

Grand Total from 3 Sites: Status at the End of this Month:	Timepoints					
	BL	6M	12M	18M	24M	Total
A. Total # of <u>subjects</u> with <u>new</u> samples/records received in the lab:	14	0	0	0	0	14
B. Total # of <u>subjects</u> whose samples/records have <u>completed</u> processing:	14	0	0	0	0	14
C. Total # of <u>subjects</u> whose samples/records are awaiting completion:	0	0	0	0	0	0
D: Total # of <u>subjects</u> whose samples/records have errors, problems, or queries, preventing processing from being completed:	0	0	0	0	0	0

Explanations:

A. This is a count of the number of subjects with new samples / records that have been forwarded from the sites this month.
Do not count replacement samples or revisions of old records in your count.

B. This is a count of subjects whose samples / records have completed processing in the lab this month. Include samples / records received this month as well as any samples / records which had been backlogged from previous months.

C. This is a count of the number of subjects whose samples / records have not completed processing and are backlogged to the next month
Include samples / records received this month as well as any samples / records backlogged from previous months.

D. From the total in C, enter the number of subjects whose processing has not been completed due to errors, problems and queries.
Do not include subjects whose samples / records have not started processing or whose processing is proceeding normally.

Monthly Data					Cumulative Data	
	A. No. subjects with new samples received:	B. No. subjects whose samples completed processing:	C. No. subjects whose samples are backlogged:	D. No. subjects whose samples have errors:	A. Cum. no. subjects with samples received:	B. Cum. no. subjects whose samples completed processing:
May-07	0	0	0	0	0	0
Jun-07	1	1	0	0	1	1
Jul-07	7	7	0	0	8	8
Aug-07	9	9	0	0	17	17
Sep-07	12	12	0	0	29	29
Oct-07	14	14	0	0	43	43
Nov-07						
Dec-07						
Jan-08						
Feb-08						
Mar-08						
Apr-08						
May-08						
Jun-08						
Jul-08						
Aug-08						
Sep-08						
Oct-08						
Nov-08						
Dec-08						

B. Quarterly Vermont Core Lab QC Reports

The following Reports will be submitted for each site quarterly to the CALERIE Coordinating Center:

CALERIE Blood QC Monitoring Summary: Shipping and Packaging

Lab: Vermont Core lab

**Review Period: September 1 - November 30,
2007**

Site: PBRC

Shipping and Packaging	Current	Cumulative
Notification and delivery not on time	0	0
Shipping container damaged or improperly labeled	0	0
Insufficient dry ice	0	0
Samples thawed	0	0
Not packaged according to IATA regulations	0	0
Aliquots incorrectly organized	0	0

Calerie Blood QC Monitoring Summary: Shipping and Packaging

Lab: Vermont Core lab

**Review Period: September 1 - November 30,
2007**

Site: PBRC

Shipping and Packaging	Current	Cumulative
-------------------------------	----------------	-------------------

Notification and delivery not on time	0	0
Shipping container damaged or improperly labeled	0	0
Insufficient dry ice	0	0
Samples thawed	0	0
Not packaged according to IATA regulations	0	0
Aliquots incorrectly organized	0	0

Calerie Blood QC Monitoring Summary: Off Cycle

Lab: Vermont Core lab

Review Period: September 1 - November 30, 2007

Site: PBRC

	Current	Cumulative
Number of shipments received @ LCBR	3	6
Number of shipments received thawed	0	0

Off-cycle Sex Hormone Collection (women only)	BaseLine	12 Month	24 Month
Number of sample sets received	3	0	0

Phlebotomy and Processing	Female Off-cycle Annual Visit Collections Combined	
	Off-Cycle Visit Day 1	Off-Cycle Visit Day 2
Missing or incomplete forms	1	0
Collection tubes not filled	2	0
Blood collection time exceeds acceptable limits	0	
Number of tubes > 90 min serum processing time	0	0
Missing aliquots or tubes upon receipt at LCBR	2	0
Aliquots not frozen upright	0	0

Calerie Blood QC Monitoring Summary: 3, 6, 18M

Lab: Vermont Core lab

Review Period: September 1 - November 30, 2007

Site: PBRC

	Current	Cumulative
Number of shipments received @ LCBR	3	6
Number of shipments received thawed	0	0

	3 Month	6 Month	18 Month
Number of sample sets received	4	0	0

Phlebotomy and Processing	Counts of Protocol Deviations - 3M, 6M, and 18M Collections Combined
Missing or incomplete forms	0
Collection tubes not filled	0
Number of tubes > 30 min plasma processing time	0
Number of tubes > 90 min serum processing time	0
Missing aliquots or tubes upon receipt at LCBR	0
Aliquots not frozen upright	0

Calerie Blood QC Monitoring Summary: 17, 23, Unscheduled

Lab: Vermont Core lab

Review Period: September 1 - November 30, 2007

Site: PBRC

	Current	Cumulative
Number of shipments received @ LCBR	3	6
Number of shipments received thawed	0	0

	17 Month	23 Month	Unscheduled (women only)
Number of sample sets received	0	0	0

Phlebotomy and Processing	Counts of Protocol Deviations - 17, 23M and Unscheduled Collections Combined
Missing or incomplete forms	0
Collection tubes not filled	0
Number of tubes > 90 min serum processing time	0
Missing aliquots or tubes upon receipt at LCBR	0
Aliquots not frozen upright	0

Calerie Tissue QC Monitoring Summary: Annual Visits			
Lab: Vermont Core lab			
Review Period: September 1 - November 30, 2007			
Site: PBRC			
	Current	Cumulative	
Number of shipments received @ LCBR	2	4	
Number of shipments received thawed	0	0	
	Baseline	12 Months	24 Months
Number of sample sets received	8	0	0
Phlebotomy and Processing	Counts of Protocol Deviations - Combined		
Missing or incomplete forms	0		
Biospsy Cryovials not filled	17		
Missing cryovials or cassettes upon receipt at LCBR	0		
Tissue IHC cassettes not received at 15-30°C	0		

IX. Import of Assay Results to the CALERIE Sample Management Database

The following steps are completed by the CALERIE Project Manager.

A. Receiving Assay Results from FAHC

Once FAHC has completed the monthly assays requested both hardcopies and an electronic result file will be sent to the LCBR. Hardcopy reports will be mailed from the FAHC Chemistry Lab via intercampus mail to Rebekah. Electronic results files will be emailed with daily Lab Reports from FAHC to the LCBR lab report recipients: Nicole, Sarah, Rebekah and Elaine. Double click on GCAL9 report and save file to: L:\Groups\LCBR\Databases\CALERIE\AssayResults

Compare electronic results files to test request file to make sure that all results expected were received. If there are any problems with the electronic results file (i.e any results are missing, CBAL numbers are not reported with results, etc) email Linda Scotti linda.scotti@vtmednet.org (cc: Monica Sullivan monica.sullivan@vtmednet.org) to see if corrected electronic file can be sent. If a corrected report cannot be obtained, results from these reports will have to manually be entered. (see section C. Manual Data Entry).

Example of electronic result file from FAHC:

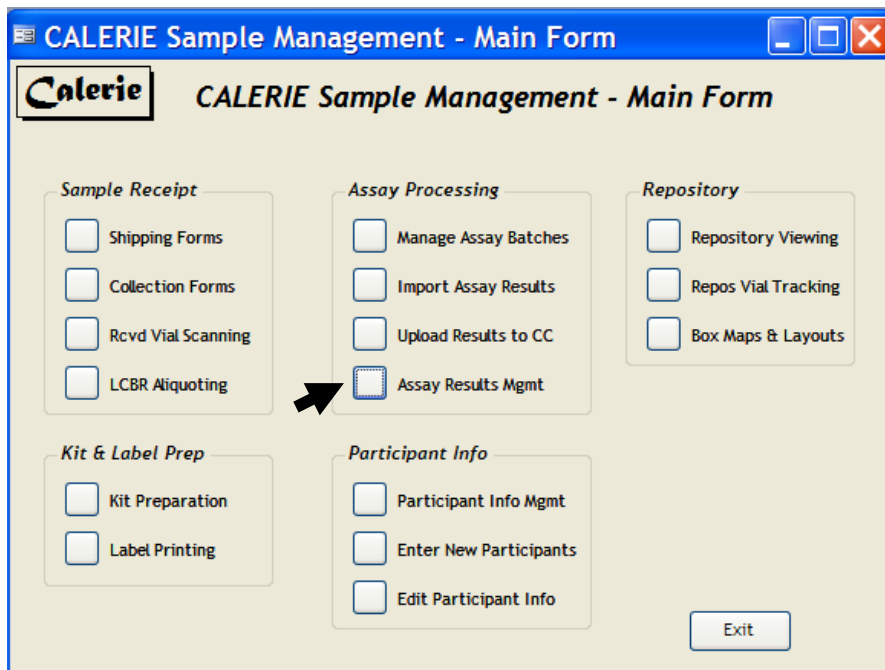
```
10580054,5/20/2008,T55387,T3,146
10580054,5/20/2008,T55387,TSH,0.38
20410054,5/20/2008,T55390,T3,99
20410054,5/20/2008,T55390,TSH,0.76
777754,5/20/2008,T55436,T3,153
777754,5/20/2008,T55436,TSH,1.23
888854,5/20/2008,T55491,T3,283
888854,5/20/2008,T55491,TSH,16.09
```

B. Importing of Electronic Results files from FAHC

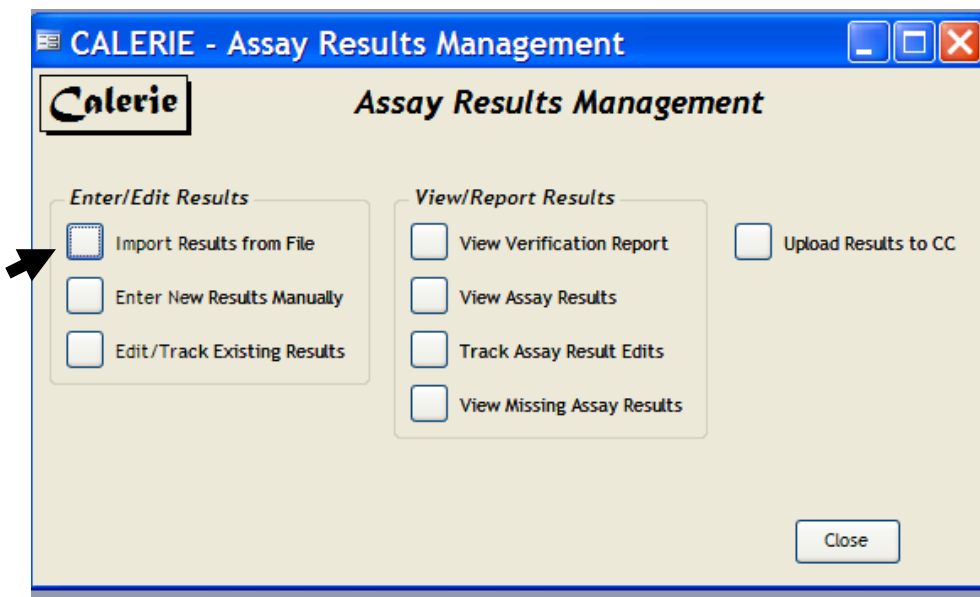
The following steps are completed by the CALERIE Project Manager.

When the result file is ready to import, open the CALERIE Sample Management database located at: L:\LCBR\Databases\CALERIE\CALERIESampleMgmt_MED34_(most recent version date)..adp

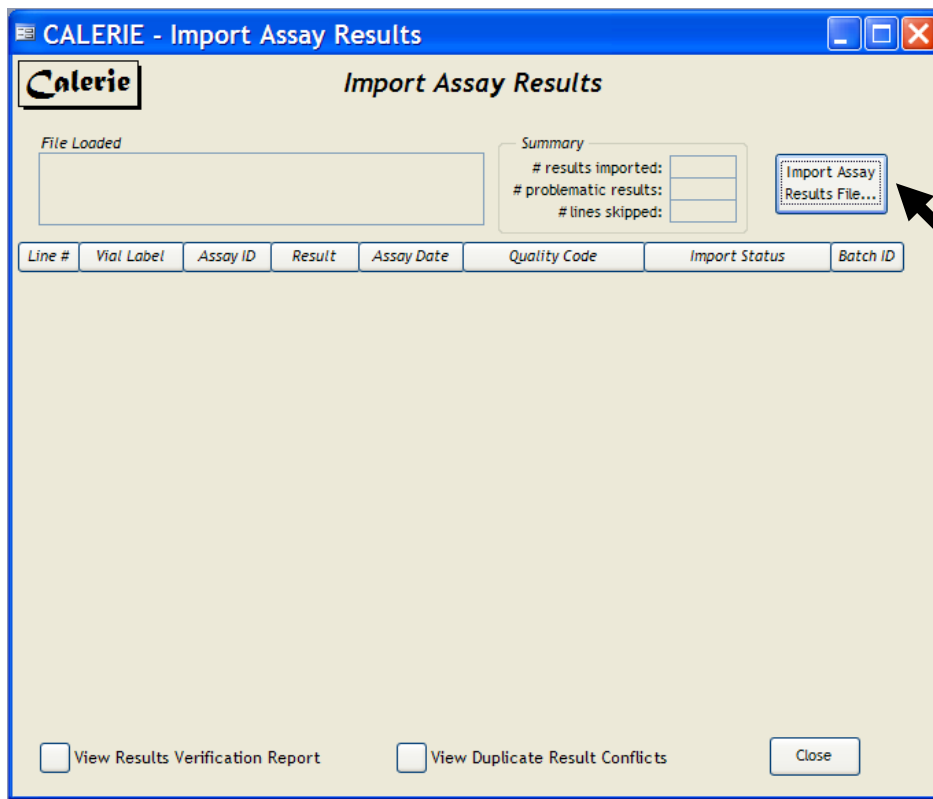
1) At the *Main Form*, select *Assay Results Mgmt* under *Assay Processing*



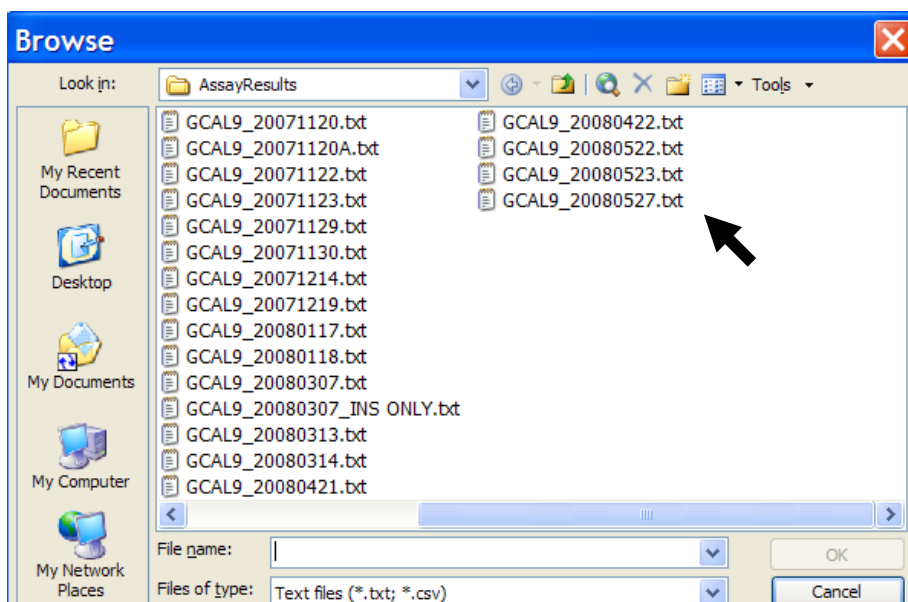
2) At the *Assay Results Management* screen, select *Import Results from File*.



3) At the *Import Assay Results* screen, select *Import Assay Results File...*



4) The assay file browser window will open. Highlight the appropriate file and click *OK*.



5) All recognizable results files will be imported into the database and will now appear in the *Import Assay Results* screen. If controls are included in the electronic file these results lines will be

not be imported and show as lines skipped in this table. (control results are manually entered into Excel. See Section D. *Serum Control Entry and Verification*)

CALERIE - Import Assay Results

Calerie

Import Assay Results

File Loaded

L:\Groups\LCBR\Databases\CALERIE\AssayResults\GCAL9_20080522.txt

Summary

results imported: 51

problematic results: 0

lines skipped: 12

Import Assay Results File...

Line #	Vial Label	Assay ID	Result	Assay Date	Quality Code	Import Status	Batch ID
1	1058 00 54	T3	146	5/20/2008	Normal result	Imported	135
2	1058 00 54	TSH	0.38	5/20/2008	Normal result	Imported	135
3	2041 00 54	T3	99	5/20/2008	Normal result	Imported	135
4	2041 00 54	TSH	0.76	5/20/2008	Normal result	Imported	135
5	2042 00 54	T3	99	5/20/2008	Normal result	Imported	135
6	2042 00 54	TSH	0.89	5/20/2008	Normal result	Imported	135
7	2037 00 54	T3	94	5/20/2008	Normal result	Imported	135
8	2037 00 54	TSH	1.74	5/20/2008	Normal result	Imported	135
9	2039 00 54	T3	130	5/20/2008	Normal result	Imported	135
10	2039 00 54	TSH	2.38	5/20/2008	Normal result	Imported	135
11	3051 00 54	T3	112	5/20/2008	Normal result	Imported	135
12	3051 00 54	TSH	2.00	5/20/2008	Normal result	Imported	135
13	3052 00 54	T3	93	5/20/2008	Normal result	Imported	135
14	3052 00 54	TSH	3.81	5/20/2008	Normal result	Imported	135
15	3053 00 54	T3	131	5/20/2008	Normal result	Imported	135
16	3053 00 54	TSH	1.09	5/20/2008	Normal result	Imported	135

☐ View Results Verification Report

☐ View Duplicate Result Conflicts

Close

C. Verification of Results Electronic Results Files

The following steps are completed by the CALERIE Project Manager.

6) Once you have successfully imported an electronic results file, select *View Results Verification Report* at the bottom left of the *Import Assay Results* table. A Results Verification Report will appear with all the results included in the electronic file that was just imported.

Calerie		Assay Results Verification		Verified by: _____ Date: _____			
Vial Label	AssayCode	AssayID	Result	Units	Assay Date	Quality Code	Assay Description
Assay Batch ID		129	Batch Type		Cortisol		
1058 00 51	CORT	CORT	23	ug/dL	5/21/2008	1 - Normal result	Cortisol
2037 00 51	CORT	CORT	5	ug/dL	5/21/2008	1 - Normal result	Cortisol
2039 00 51	CORT	CORT	11	ug/dL	5/21/2008	1 - Normal result	Cortisol
2041 00 51	CORT	CORT	14	ug/dL	5/21/2008	1 - Normal result	Cortisol
2042 00 51	CORT	CORT	5	ug/dL	5/21/2008	1 - Normal result	Cortisol
3051 00 51	CORT	CORT	10	ug/dL	5/21/2008	1 - Normal result	Cortisol
3052 00 51	CORT	CORT	12	ug/dL	5/21/2008	1 - Normal result	Cortisol
3053 00 51	CORT	CORT	12	ug/dL	5/21/2008	1 - Normal result	Cortisol
3054 00 51	CORT	CORT	13	ug/dL	5/21/2008	1 - Normal result	Cortisol
3055 00 51	CORT	CORT	15	ug/dL	5/21/2008	1 - Normal result	Cortisol
Assay Batch ID		131	Batch Type		DHEA-Sulfate,Insulin		
1058 00 56	DHES	DHES	118	ug/dL	5/20/2008	1 - Normal result	DHEA-S
2037 00 56	DHES	DHES	73	ug/dL	5/20/2008	1 - Normal result	DHEA-S
2039 00 56	DHES	DHES	27	ug/dL	5/20/2008	1 - Normal result	DHEA-S
2041 00 56	DHES	DHES	102	ug/dL	5/20/2008	1 - Normal result	DHEA-S
2042 00 56	DHES	DHES	131	ug/dL	5/20/2008	1 - Normal result	DHEA-S
3051 00 56	DHES	DHES	110	ug/dL	5/20/2008	1 - Normal result	DHEA-S
3052 00 56	DHES	DHES	122	ug/dL	5/20/2008	1 - Normal result	DHEA-S
3053 00 56	DHES	DHES	77	ug/dL	5/20/2008	1 - Normal result	DHEA-S
3054 00 56	DHES	DHES	122	ug/dL	5/20/2008	1 - Normal result	DHEA-S
3055 00 56	DHES	DHES	181	ug/dL	5/20/2008	1 - Normal result	DHEA-S
Assay Batch ID		132	Batch Type		LH,FSH,FTES		
2041 00 52	SHBG	SXBG	21.5	nmol/L	5/20/2008	1 - Normal result	Sex Hormone Binding Globulin
2042 00 52	SHBG	SXBG	16.3	nmol/L	5/20/2008	1 - Normal result	Sex Hormone Binding Globulin
Assay Batch ID		134	Batch Type		Progesterone		
1058 00 42	PROGD2	PROG	2.7	ng/mL	5/21/2008	1 - Normal result	Progesterone
1058 00 52	PROG0	PROG	2.9	ng/mL	5/21/2008	1 - Normal result	Progesterone
2037 00 52	PROG0	PROG	0.4	ng/mL	5/21/2008	1 - Normal result	Progesterone
2039 00 52	PROG0	PROG	0.2	ng/mL	5/21/2008	1 - Normal result	Progesterone
3051 00 52	PROG0	PROG	0.9	ng/mL	5/21/2008	1 - Normal result	Progesterone
3052 00 52	PROG0	PROG	13.9	ng/mL	5/21/2008	1 - Normal result	Progesterone

5/30/2008 10:05:36 AM Page 1 of 2

7) Reports are filed by test, so print a verification report to attach to each test report group. At the bottom of each page write “entered by:_____” (initials of person who imported results) “Date:_____” (date results were entered)”. Give the verification report and forms to another technician to verify results.

8) Once results are verified, data need to be reviewed by Michael Lewis or Elaine Cornell. Once data is approved for transmit to Duke, Michael or Elaine will sign and date the CALERIE Data Transmit Approval signature form (Appendix E).

D. Manual Entry of Hardcopy Results from FAHC

The following steps are completed by the CALERIE Project Manager.

1) At the *Main Form*, select *Assay Results Mgmt* under *Assay Processing*

CALERIE Sample Management - Main Form

Calerie **CALERIE Sample Management - Main Form**

Sample Receipt

- ☐ Shipping Forms
- ☐ Collection Forms
- ☐ Rcvd Vial Scanning
- ☐ LCBR Aliquoting

Assay Processing

- ☐ Manage Assay Batches
- ☐ Import Assay Results
- ☐ Upload Results to CC
- ☒ Assay Results Mgmt

Repository

- ☐ Repository Viewing
- ☐ Repos Vial Tracking
- ☐ Box Maps & Layouts

Kit & Label Prep

- ☐ Kit Preparation
- ☐ Label Printing

Participant Info

- ☐ Participant Info Mgmt
- ☐ Enter New Participants
- ☐ Edit Participant Info

Exit

2) Select *Enter New Results Manually* from the *Enter/Edit Results* Menu on the left.

CALERIE - Assay Results Management

Calerie **Assay Results Management**

Enter/Edit Results

- ☐ Import Results from File
- ☒ Enter New Results Manually
- ☐ Edit/Track Existing Results

View/Report Results

- ☐ View Verification Report
- ☐ View Assay Results
- ☐ Track Assay Result Edits
- ☐ View Missing Assay Results

☐ Upload Results to CC

Close

3) The *Assay Result Manual Entry* screen will display. Type the first participant vial id number into the *Vial Label* entry box. Click on the drop-down menu under *Assay ID* and select the first assay result you are entering.

CALERIE - Assay Result Manual Entry

Calerie *Assay Result Manual Entry*

Vial Label: 1005 00 52 Quality Code: Normal result

Assay ID: Assay Result:

Assay Code: PROG Assay Date: 7/28/2007

Comment:

Import Status:

Save This Result Close

4) The quality code will default to normal result. If the result is coded (-333, -444, -777, etc), select the appropriate quality code from the options.

CALERIE - Assay Result Manual Entry

Calerie *Assay Result Manual Entry*

Vial Label: 1005 00 52 Quality Code: Normal result

Assay ID: PROG Assay Result:

Assay Code: PROG0 Assay Date:

Comment:

Import Status:

Save This Result Close

Quality Code dropdown options:

- Unknown
- Normal result
- No sample
- Quantity insufficient for assay
- Poor quality - no result
- Poor quality - result obtained
- Out of range - low value
- Out of range - high value
- Off-cycle exclusion

LCBR Code	Definition
-999	No sample
-888	Quantity not sufficient for assay
-444	Poor quality-no result (enter result if obtained; -444 if no result obtained)
-333	Out of range-low value
-555	Out of range-high value

5) Enter the assay result code or result, the assay date, and any comments into the appropriate boxes. Select *Save This Result* once you are complete. Continue steps 3-5 for all data that needs to manually be entered.

6) Once all data has been entered, return to the *Assay Results Management Main Form*. Click review verification report. See section **C. Verification of Results Electronic Results Files** And repeat these steps for the data just entered.

E. Entry of Serum Control Data from FAHC

The following steps are completed by the CALERIE Project Manager.

Both electronic and hardcopy results of serum set, high and low controls will be included with study sample result reports. Since the controls are tracked separately from the database, the hardcopy and not the electronic result reports are utilized for entry of these results.

1.) Separate out the control hardcopy reports from the study sample reports. If this is a serum set month separate those out as well.

2.) Open the control entry excel file *CALERIE_LCBR Serum Controls to FAHC.xls* saved in: L:\Groups\LCBRSecure\Excel\CAalerie\Data\Internal and FAHC QC.

3.) Enter high and low controls into the first worksheet “Hi-Lo_Controls”. Record the assay date in the first column and the individual test results in the appropriate columns for each control.

4.) Once you have entered the high and low controls, proceed to the serum set control entry. Each set of test results for the 20 samples is recorded into an individual worksheet. Click on the appropriate test worksheet and enter the results for serum set ids 1-20. Proceed to the next test until all tests have been entered. If any tests that were expected are missing from the hardcopy reports, email Monica Sullivan (Monica.Sullivan@vtmednet.org) for assistance.

5) The second worksheet of this workbook, named “Serum Sets” is a cumulative file of all the results for all tests and test dates. Make a new column for the current assay date for each test after the previous test date. Copy and paste the newly entered results for each test into this file from each individual worksheet.

6.) Print a copy of the serum “Hi-Lo_Controls” worksheet for verification. In the Serum Sets worksheet hide all columns for results on previous test dates. Once only results for the current test date are displayed, print a copy of the current serum set data for verification.

7.) Ask another technician to verify your entry of these control data. Make any edits where applicable to the Excel file and file in the control set folders in with the FAHC results files (located in the CALERIE file cabinet in T208, Rebekah’s office).

8) Rebekah will email control file to Elaine and Michael for review.

9) Once data is reviewed and accepted, Michael Lewis or Elaine Cornell will sign and date the CALERIE Quality Control Data Approval signature form.

F. Quarterly Control Data from FAHC

The following steps are completed by the CALERIE Project Manager.

Once every 3 months we request FAHC internal control data for the tests run in the CALERIE study.

1.) Rebekah will email Monica Sullivan the last version of the Excel spreadsheet “CALERIE FAHC QC data_(last date saved)” located in:

L:\Groups\LCBRSecure\Excel\Calerie\Data\Internal and FAHC QC

2.) Monica enters the new controls for the currently ending quarter and will email the file with a new saved date in the file title.

3.) Rebekah saves this file in the folder with the previous FAHC QC files and emails to Michael and Elaine to review.

4) Once data is reviewed and accepted, Michael Lewis or Elaine Cornell will sign and date the CALERIE Quality Control Data Approval signature form.

X. ELECTRONIC TRANSFER OF RESULTS TO THE COORDINATING CENTER

The following steps are completed by the CALERIE Project Manager.

A. The Assay Results Transmission Batches Screen

Bring up the Assay Results Transmission Batches form – you can get there from the Main Form or the Assay Results Management screen via the Upload Results to CC button.

The “results” that appear on this screen don’t actually come directly from the assay results table; rather, they are drawn from the assay results *audit* table. Thus, on this screen we are actually looking at *audit records* of changes made to the assay results table. The reasoning here is that if an assay result record is altered for any reason, we want the option to transmit that change to the coordinating center.

Batch Actions

- ☐ Create a New Xmit Batch
- ☐ Create the Results Upload File
- ☐ View the Upload File
- ☐ Transmit Results to Coord Center
- ☐ Notify Coord Center of Upload

Specify Unbatched Result Edits to View

CALERIE ID: [] Visit ID: [] Assay Code: [] Assay Date: 1/17/2008

☒ On-hold edits ☐ Hidden edits

Unbatched Result Edits

AuditID	PptID	VialLabel	AssayCode	Result	AssayDate	Qual	Activity	Timestamp	Comr
1176	01-0096	1044 00 50	CHHDLR	4.9	1/17/2008	1	New	1/18/2008 2:13:32 PM	
1177	01-0096	1044 00 50	CHOL	248	1/17/2008	1	New	1/18/2008 2:13:32 PM	
1178	01-0096	1044 00 50	HDL	51	1/17/2008	1	New	1/18/2008 2:13:32 PM	
1179	01-0096	1044 00 50	LDL	168	1/17/2008	1	New	1/18/2008 2:13:32 PM	
1226	01-0096	1044 00 55	SGL0	87	1/17/2008	1	New	1/18/2008 2:13:35 PM	
1180	01-0096	1044 00 50	TRIG	145	1/17/2008	1	New	1/18/2008 2:13:32 PM	
1181	01-0097	1032 00 50	CHHDLR	2.9	1/17/2008	1	New	1/18/2008 2:13:32 PM	
1182	01-0097	1032 00 50	CHOL	135	1/17/2008	1	New	1/18/2008 2:13:32 PM	
1183	01-0097	1032 00 50	HDL	47	1/17/2008	1	New	1/18/2008 2:13:32 PM	
1184	01-0097	1032 00 50	LDL	80	1/17/2008	1	New	1/18/2008 2:13:32 PM	

Batch Selection

Batch	XmitDate	XmitFile
5	1/17/2008	2008-Jan\AssayResults_00
4	1/17/2008	2008-Jan\AssayResults_00
3	1/17/2008	2008-Jan\AssayResults_00
2	1/17/2008	2008-Jan\AssayResults_00
1	11/28/2007	2007-Nov\AssayResults_00

Result Edits in Selected Batch

AuditID	PptID	VialLabel	AssayCode	Result	AssayDate	Qual	Activity	Timestamp	Comr
750	01-0007	1017 00 55	SGL0	82	10/17/2007	1	New	11/1/2007 10:54:27 AM	
749	01-0007	1017 00 41	SGL120	137	10/17/2007	1	New	11/1/2007 10:54:27 AM	
747	01-0007	1017 00 38	SGL30	120	10/17/2007	1	New	11/1/2007 10:54:27 AM	
807	01-0007	1017 00 39	SGL60	102	10/17/2007	1	New	11/1/2007 10:54:30 AM	
748	01-0007	1017 00 40	SGL90	125	10/17/2007	1	New	11/1/2007 10:54:27 AM	
700	01-0024	1005 00 55	SGL0	83	9/28/2007	1	New	10/24/2007 10:34:30 AM	
699	01-0024	1005 00 41	SGL120	126	9/28/2007	1	New	10/24/2007 10:34:18 AM	
697	01-0024	1005 00 38	SGL30	128	9/28/2007	1	New	10/24/2007 10:33:54 AM	

The *Batch Selection* listbox displays the transmission batches that have been created. When a batch is selected, the results assigned to it appear in the *Result Edits in Selected Batch* listbox.

The *Unbatched Result Edits* listbox is used to display results (or edits thereof) that have not been transmitted to the coordinating center. This is initially empty when the screen is opened. It gets populated by specifying criteria in the *Specify Unbatched Result Edits to View* region and pressing the *Retrieve Results* button.

A note about On-hold edits versus Hidden edits. *On-hold edits* are result audit records that are pending review for transmission. *Hidden edits* are those that are not to be considered for transmission. Normally, when a result is imported or manually edited, that audit record is marked as On-hold (and any previous on-hold edits to that result are automatically flagged as hidden – the assumption being that only the most recent version of a result record should be transmitted). There are some exceptions, the most notable being off-cycle exclusions – these always get marked as hidden.

In either of these result listboxes, double-clicking a row brings up the Assay Result Tracking/Editing screen, which shows the result as currently recorded in the database together with a history of all audit records associated with that result. For unbatched results, this provides a place to manually toggle between On-hold and Hidden status. [This is clunky interface, would like to improve it, but this operation normally isn't needed –the right thing usually happens. --DED]

Results are added to or removed from a batch via the up- and down-arrow buttons. A warning is put up if the batch has an associated XmitDate or XmitFile – the idea being that a change to such a batch will invalidate these data. (Double-clicking an item in the *Batch Selection* listbox brings up screen where these can be edited.)

B. Preparing a Transmit Batch

The procedure of creating and transmitting a batch of assay results to the coordinating center is guided by the Batch Actions section of the Assay Results Transmission Batches screen. A brief summary of the procedure is:

1. Create and prepare a results transmission batch.
2. Create the corresponding results upload file.
3. Transmit the upload file.
4. Notify the coordinating center via email that a results file has been uploaded.

In greater detail, a typical upload session might go as follows. Begin by clicking the Create a New Xmit Batch button. A new batch appears and is selected in the Batch Selection listbox.

Enter criteria specifying which unbatched results you wish to consider and click Retrieve Results. (If you wish to view *all* on-hold edits, just leave these fields blank with On-hold edits selected.) From the Unbatched Result Edits listbox, select which results you wish to include in the new batch and click the down-arrow button to move them.

Once you are satisfied that the new batch contains the results that you want, it is time to create the results text file that actually gets uploaded to the coordinating center. Click *Create the Results Upload File* to accomplish this. This file should be saved somewhere within the folder L:\Groups\LCBR\Databases\CALERIE\FilesToCC. Although you may name this file whatever you want, it is probably best practice to stick with the suggested filename. Once saved, you can click the *View the Upload File* if you wish to look at the file in NotePad.

Note: It is suggested to decide on conventions for the naming and location of these upload files. Once saved, it is best to not move them, for that will invalidate the *XmitFile* field in the *Batch Selection* listbox, and the *View the Upload File* button won't work (this link can be reestablished by double-clicking the batch in the Batch Selection listbox).

C. Uploading and Transmitting a Batch to Duke

At this point, you are ready to upload the file. Click *Transmit Results to Coord Center*. This will start a command-line script called UploadToDuke.bat that opens a connection to the Duke upload site via the scp2 command (part of the SSH Secure Shell software). You will be prompted for a

password. After correctly entering the password, the file will be uploaded. Wait for the script to finish. It should reply with either Upload succeeded or Uploading terminated with errors. The CALERIE front-end will be asking you whether the file was successfully uploaded – answer appropriately. If it was successful, the batch’s XmitDate field will be set to today’s date.

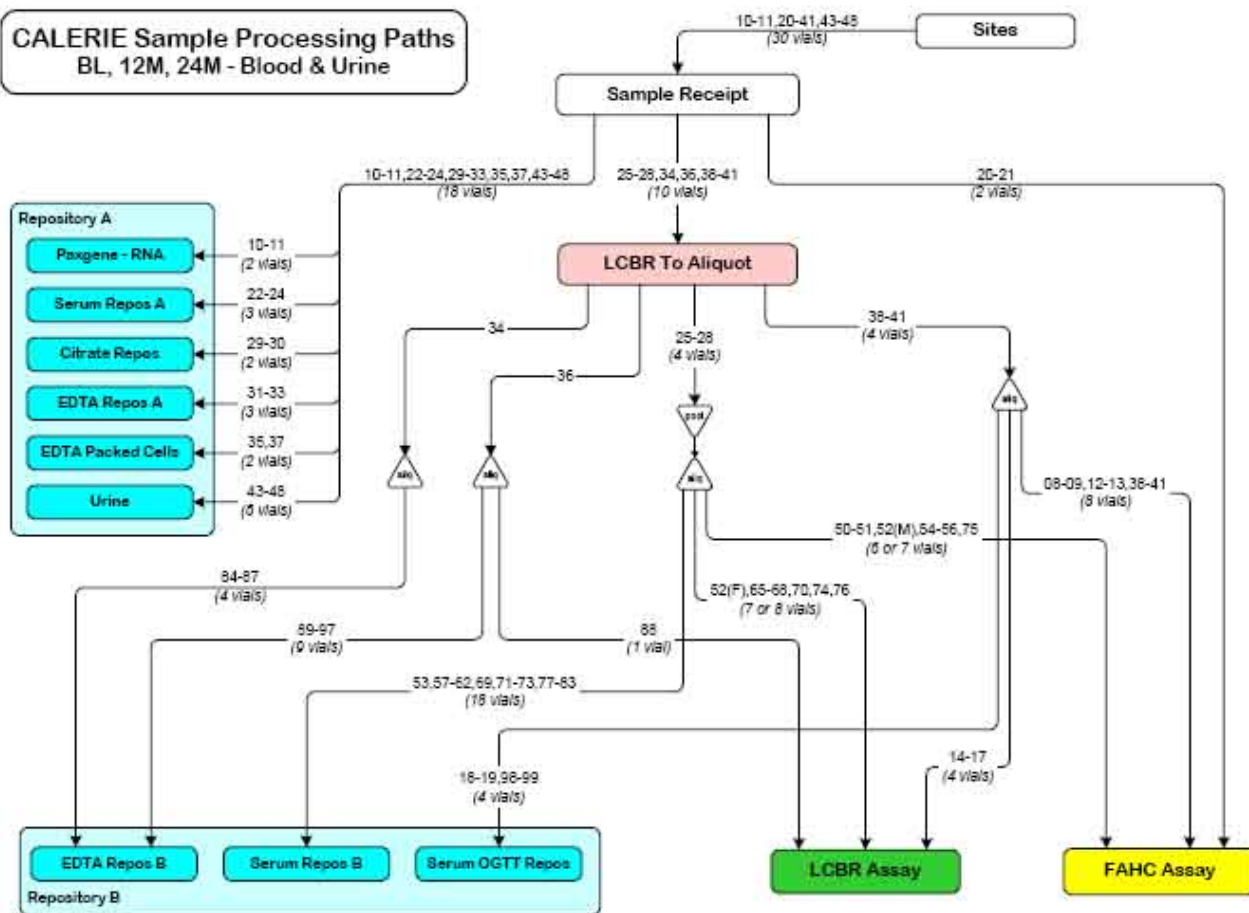
Finally, it remains to notify Duke that a file has been uploaded. Click *Notify Coord Center of Upload* – this will bring up an email message formatted appropriately. Feel free to edit or add any comments to the message. Click *Send* when you are ready to send the message (or you can cancel the message if you wish; you are not committed to actually sending the message).

Once transmit is complete, Project Manager signs and dates the Study Data Transmit Signature form (Appendix E). Files are now ready to file in file cabinet in T227B (Rebekah’s office)

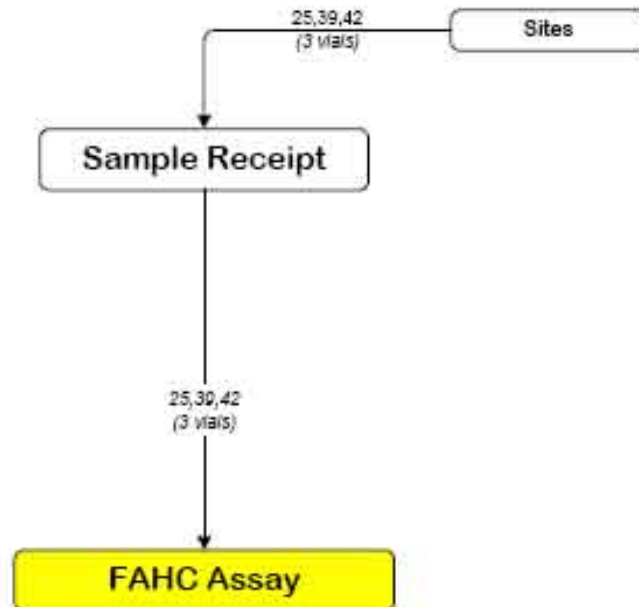
XI. APPENDIX

APPENDIX A. Schematics of LCBR Aliquoting Scheme

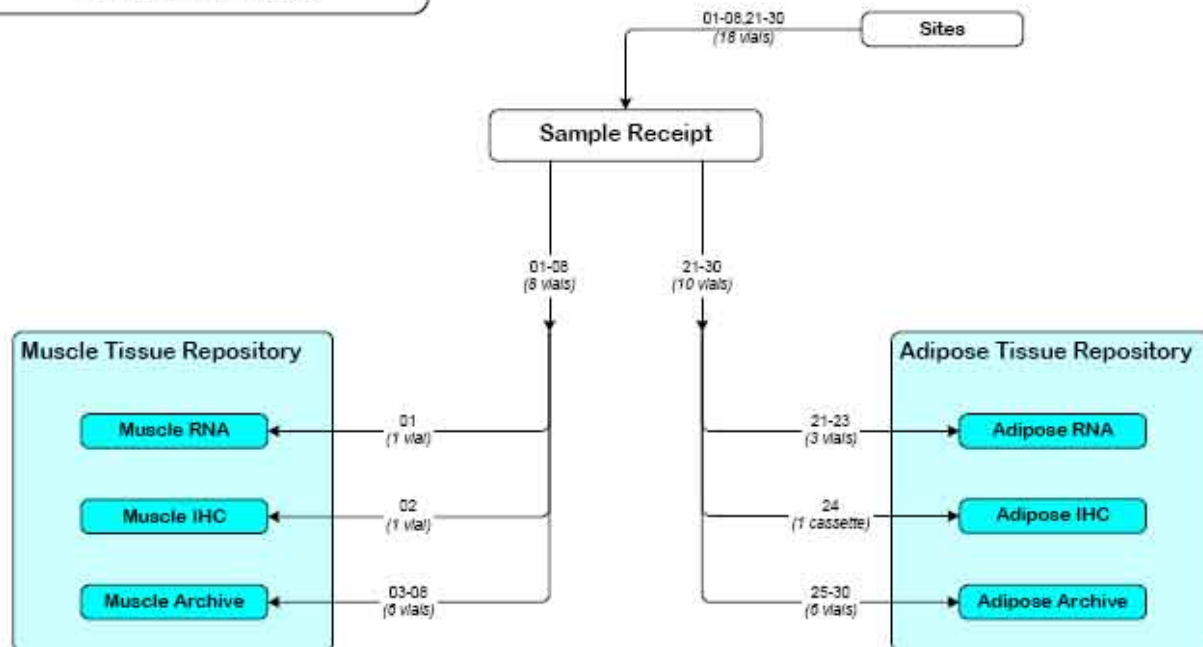
CALERIE Sample Processing Paths BL, 12M, 24M - Blood & Urine



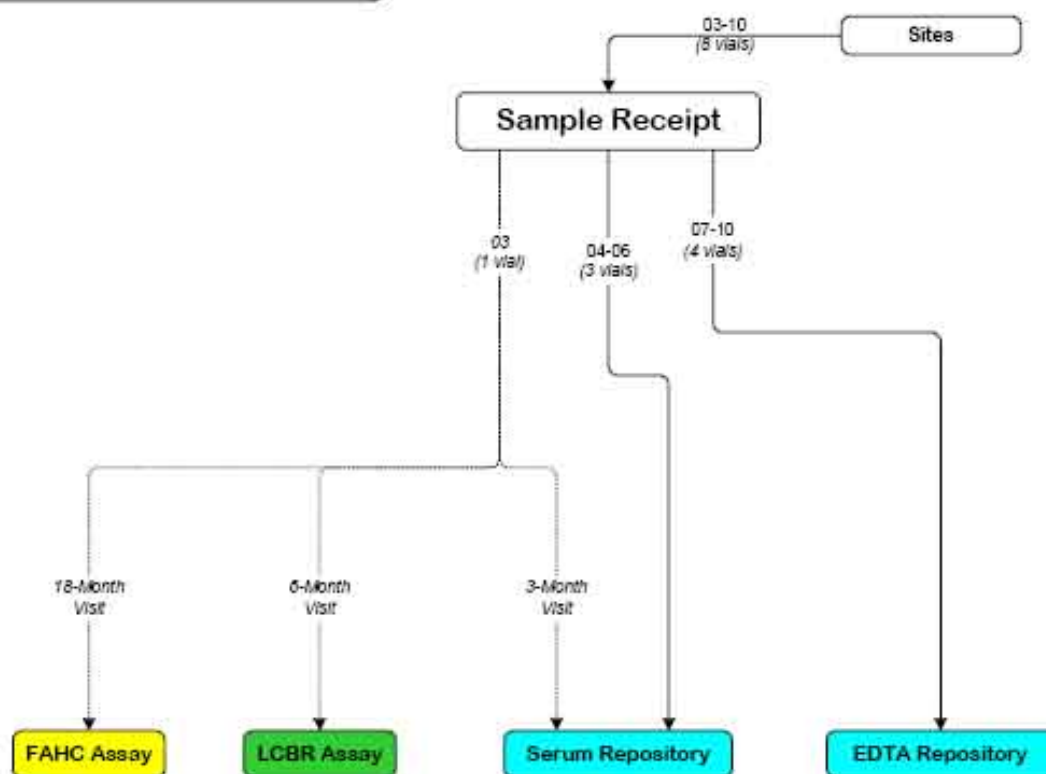
CALERIE Sample Processing Paths
BL, 12M, 24M - Off-Cycle



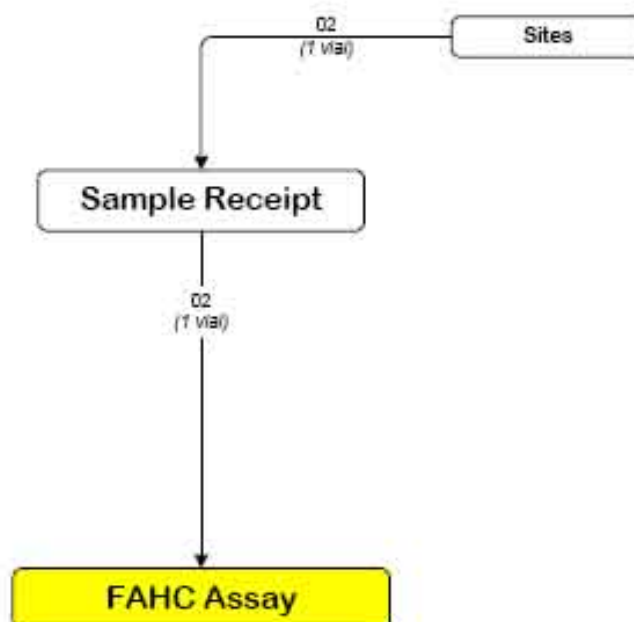
CALERIE Sample Processing Paths
BL, 12M, 24M - Tissue



CALERIE Sample Processing Paths
3-Month, 6-Month, 18-Month



CALERIE Sample Processing Paths
17-Month, 23-Month, Unscheduled



APPENDIX B. Testing and Repository Box Maps

B.1 SAMPLE RECEIPT BOXES

FAHC-Mayo Testing (Catecholamines)

EDTA Plasma - 10ml Tubes (# 20 & 21)

PT 1 cryo 20	PT 4 cryo 21	PT 8 cryo 20	PT 11 cryo 21	PT 15 cryo 20	PT 18 cryo 21	PT 22 cryo 20
PT 1 cryo 21	PT 5 cryo 20	PT 8 cryo 21	PT 12 cryo 20	PT 15 cryo 21	PT 19 cryo 20	PT 22 cryo 21
PT 2 cryo 20	PT 5 cryo 21	PT 9 cryo 20	PT 12 cryo 21	PT 16 cryo 20	PT 19 cryo 21	PT 23 cryo 20
PT 2 cryo 21	PT 6 cryo 20	PT 9 cryo 21	PT 13 cryo 20	PT 16 cryo 21	PT 20 cryo 20	PT 23 cryo 21
PT 3 cryo 20	PT 6 cryo 21	PT 10 cryo 20	PT 13 cryo 21	PT 17 cryo 20	PT 20 cryo 21	PT 24 cryo 20
PT 3 cryo 21	PT 7 cryo 20	PT 10 cryo 21	PT 14 cryo 20	PT 17 cryo 21	PT 21 cryo 20	PT 24 cryo 21
PT 4 cryo 20	PT 7 cryo 21	PT 11 cryo 20	PT 14 cryo 21	PT 18 cryo 20	PT 21 cryo 21	X

FAHC-Mayo Testing (CPEP)

EDTA Plasma – Serum 5mL tube #75 (time 0 serum) and tubes 8, 9, 12, and 13 (OGTT sera)

PT 1 tube 8	PT 2 tube 8	PT 3 tube 8	PT 4 tube 8	PT 5 tube 8	PT 6 tube 8	PT 7 tube 8
PT 1 Tube 9	PT 2 Tube 9	PT 3 Tube 9	PT 4 Tube 9	PT 5 Tube 9	PT 6 Tube 9	PT 7 Tube 9
PT 1 Tube 12	PT 2 Tube 12	PT 3 Tube 12	PT 4 Tube 12	PT 5 Tube 12	PT 6 Tube 12	PT 7 Tube 12
PT 1 Tube 13	PT 2 Tube 13	PT 3 Tube 13	PT 4 Tube 13	PT 5 Tube 13	PT 6 Tube 13	PT 7 Tube 13
Ppt 1 Tube 75	Ppt 2 Tube 75	Ppt 3 Tube 75	Ppt 4 Tube 75	Ppt 5 Tube 75	Ppt 6 Tube 75	Ppt 7 Tube 75

CALERIE Annual Visit Pristine Serum Repository Cryovials

Serum- Red-top 1.5mL cryovials (Cryos #22-24)

1.0mL sample volume. 3 Cryos per participant. 33 participants per box.

Pt 1	Pt 4	Pt 7	Pt 10	Pt 13	Pt 16	Pt 19	Pt 22	Pt 25	Pt 28
cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22
Pt 1	Pt 4	Pt 7	Pt 10	Pt 13	Pt 16	Pt 19	Pt 22	Pt 25	Pt 28
cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23
Pt 1	Pt 4	Pt 7	Pt 10	Pt 13	Pt 16	Pt 19	Pt 22	Pt 25	Pt 28
cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24
Pt 2	Pt 5	Pt 8	Pt 11	Pt 14	Pt 17	Pt 20	Pt 23	Pt 26	Pt 29
cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22
Pt 2	Pt 5	Pt 8	Pt 11	Pt 14	Pt 17	Pt 20	Pt 23	Pt 26	Pt 29
cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23
Pt 2	Pt 5	Pt 8	Pt 11	Pt 14	Pt 17	Pt 20	Pt 23	Pt 26	Pt 29
cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24
Pt 3	Pt 6	Pt 9	Pt 12	Pt 15	Pt 18	Pt 21	Pt 24	Pt 27	Pt 30
cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22	cryo 22
Pt 3	Pt 6	Pt 9	Pt 12	Pt 15	Pt 18	Pt 21	Pt 24	Pt 27	Pt 30
cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23	cryo 23
Pt 3	Pt 6	Pt 9	Pt 12	Pt 15	Pt 18	Pt 21	Pt 24	Pt 27	Pt 30
cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24	cryo 24

CALERIE Annual Visit Serum 25 Tubes “LCBR to Aliquot”

Serum-10mL Transfer Tube #25

Approximately 5.0mL sample volume. 1 Tube per participant. 48 participants per box.

PT 1 cryo 25	PT 8 cryo 25	PT 15 cryo 25	PT 22 cryo 25	PT 29 cryo 25	PT 36 cryo 25	PT 43 cryo 25
PT 2 cryo 25	PT 9 cryo 25	PT 16 cryo 25	PT 23 cryo 25	PT 30 cryo 25	PT 37 cryo 25	PT 44 cryo 25
PT 3 cryo 25	PT 10 cryo 25	PT 17 cryo 25	PT 24 cryo 25	PT 31 cryo 25	PT 38 cryo 25	PT 45 cryo 25
PT 4 cryo 25	PT 11 cryo 25	PT 18 cryo 25	PT 25 cryo 25	PT 32 cryo 25	PT 39 cryo 25	PT 46 cryo 25
PT 5 cryo 25	PT 12 cryo 25	PT 19 cryo 25	PT 26 cryo 25	PT 33 cryo 25	PT 40 cryo 25	PT 47 cryo 25
PT 6 cryo 25	PT 13 cryo 25	PT 20 cryo 25	PT 27 cryo 25	PT 34 cryo 25	PT 41 cryo 25	PT 48 cryo 25
PT 7 cryo 25	PT 14 cryo 25	PT 21 cryo 25	PT 28 cryo 25	PT 35 cryo 25	PT 42 cryo 25	X

CALERIE Annual Visit Serum 26 Tubes “LCBR to Aliquot”

Serum-10mL Transfer Tube #26

Approximately 5.0mL sample volume. 1 Tube per participant. 48 participants

PT 1 cryo 26	PT 8 cryo 26	PT 15 cryo 26	PT 22 cryo 26	PT 29 cryo 26	PT 36 cryo 26	PT 43 cryo 26
PT 2 cryo 26	PT 9 cryo 26	PT 16 cryo 26	PT 23 cryo 26	PT 30 cryo 26	PT 37 cryo 26	PT 44 cryo 26
PT 3 cryo 26	PT 10 cryo 26	PT 17 cryo 26	PT 24 cryo 26	PT 31 cryo 26	PT 38 cryo 26	PT 45 cryo 26
PT 4 cryo 26	PT 11 cryo 26	PT 18 cryo 26	PT 25 cryo 26	PT 32 cryo 26	PT 39 cryo 26	PT 46 cryo 26
PT 5 cryo 26	PT 12 cryo 26	PT 19 cryo 26	PT 26 cryo 26	PT 33 cryo 26	PT 40 cryo 26	PT 47 cryo 26
PT 6 cryo 26	PT 13 cryo 26	PT 20 cryo 26	PT 27 cryo 26	PT 34 cryo 26	PT 41 cryo 26	PT 48 cryo 26
PT 7 cryo 26	PT 14 cryo 26	PT 21 cryo 26	PT 28 cryo 26	PT 35 cryo 26	PT 42 cryo 26	X

CALERIE Annual Visit Serum 27 Tubes “LCBR to Aliquot”

Serum-10mL Transfer Tube #27

Approximately 5.0mL sample volume. 1 Tube per participant. 48 participants

PT 1 cryo 27	PT 8 cryo 27	PT 15 cryo 27	PT 22 cryo 27	PT 29 cryo 27	PT 36 cryo 27	PT 43 cryo 27
PT 2 cryo 27	PT 9 cryo 27	PT 16 cryo 27	PT 23 cryo 27	PT 30 cryo 27	PT 37 cryo 27	PT 44 cryo 27
PT 3 cryo 27	PT 10 cryo 27	PT 17 cryo 27	PT 24 cryo 27	PT 31 cryo 27	PT 38 cryo 27	PT 45 cryo 27
PT 4 cryo 27	PT 11 cryo 27	PT 18 cryo 27	PT 25 cryo 27	PT 32 cryo 27	PT 39 cryo 27	PT 46 cryo 27
PT 5 cryo 27	PT 12 cryo 27	PT 19 cryo 27	PT 26 cryo 27	PT 33 cryo 27	PT 40 cryo 27	PT 47 cryo 27
PT 6 cryo 27	PT 13 cryo 27	PT 20 cryo 27	PT 27 cryo 27	PT 34 cryo 27	PT 41 cryo 27	PT 48 cryo 27
PT 7 cryo 27	PT 14 cryo 27	PT 21 cryo 27	PT 28 cryo 27	PT 35 cryo 27	PT 42 cryo 27	X

CALERIE Annual Visit Serum 28 Tubes “LCBR to Aliquot”

Serum-10mL Transfer Tube #28

Approximately 5.0mL sample volume. 1 Tube per participant. 48 participants

PT 1 cryo 28	PT 8 cryo 28	PT 15 cryo 28	PT 22 cryo 28	PT 29 cryo 28	PT 36 cryo 28	PT 43 cryo 28
PT 2 cryo 28	PT 9 cryo 28	PT 16 cryo 28	PT 23 cryo 28	PT 30 cryo 28	PT 37 cryo 28	PT 44 cryo 28
PT 3 cryo 28	PT 10 cryo 28	PT 17 cryo 28	PT 24 cryo 28	PT 31 cryo 28	PT 38 cryo 28	PT 45 cryo 28
PT 4 cryo 28	PT 11 cryo 28	PT 18 cryo 28	PT 25 cryo 28	PT 32 cryo 28	PT 39 cryo 28	PT 46 cryo 28
PT 5 cryo 28	PT 12 cryo 28	PT 19 cryo 28	PT 26 cryo 28	PT 33 cryo 28	PT 40 cryo 28	PT 47 cryo 28
PT 6 cryo 28	PT 13 cryo 28	PT 20 cryo 28	PT 27 cryo 28	PT 34 cryo 28	PT 41 cryo 28	PT 48 cryo 28
PT 7 cryo 28	PT 14 cryo 28	PT 21 cryo 28	PT 28 cryo 28	PT 35 cryo 28	PT 42 cryo 28	X

CALERIE Pristine Citrate Repository Cryovials

Citrate Plasma-Blue Top 1.5mL Cryovial (#29 &30)

1.0mL sample volume. 2 Cryos per participant, 49 participants per box.

Pt 1	Pt 6	Pt 11	Pt 16	Pt 21	Pt 26	Pt 31	Pt 36	Pt 41	Pt 46
cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29
Pt 1	Pt 6	Pt 11	Pt 16	Pt 21	Pt 26	Pt 31	Pt 36	Pt 41	Pt 46
cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30
Pt 2	Pt 7	Pt 12	Pt 17	Pt 22	Pt 27	Pt 32	Pt 37	Pt 42	Pt 47
cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29
Pt 2	Pt 7	Pt 12	Pt 17	Pt 22	Pt 27	Pt 32	Pt 37	Pt 42	Pt 47
cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30
Pt 3	Pt 8	Pt 13	Pt 18	Pt 23	Pt 28	Pt 33	Pt 38	Pt 43	Pt 48
cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29
Pt 3	Pt 8	Pt 13	Pt 18	Pt 23	Pt 28	Pt 33	Pt 38	Pt 43	Pt 48
cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30
Pt 4	Pt 9	Pt 14	Pt 19	Pt 24	Pt 29	Pt 34	Pt 39	Pt 44	Pt 49
cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29
Pt 4	Pt 9	Pt 14	Pt 19	Pt 24	Pt 29	Pt 34	Pt 39	Pt 44	Pt 49
cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30
Pt 5	Pt 10	Pt 15	Pt 20	Pt 25	Pt 30	Pt 35	Pt 40	Pt 45	X
cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	cryo 29	
Pt 5	Pt 10	Pt 15	Pt 20	Pt 25	Pt 30	Pt 35	Pt 40	Pt 45	X
cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	cryo 30	

CALERIE Annual Visit Pristine EDTA Repository Cryovials

EDTA-Purple Top 1.5mL Cryovials (#31-33)

1.0mL sample volume. 3 Cryos per participant. 33 participants per box.

Pt 1	Pt 4	Pt 7	Pt 10	Pt 13	Pt 16	Pt 19	Pt 22	Pt 25	Pt 28
cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31
Pt 1	Pt 4	Pt 7	Pt 10	Pt 13	Pt 16	Pt 19	Pt 22	Pt 25	Pt 28
cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32
Pt 1	Pt 4	Pt 7	Pt 10	Pt 13	Pt 16	Pt 19	Pt 22	Pt 25	Pt 28
cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33
Pt 2	Pt 5	Pt 8	Pt 11	Pt 14	Pt 17	Pt 20	Pt 23	Pt 26	Pt 29
cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31
Pt 2	Pt 5	Pt 8	Pt 11	Pt 14	Pt 17	Pt 20	Pt 23	Pt 26	Pt 29
cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32
Pt 2	Pt 5	Pt 8	Pt 11	Pt 14	Pt 17	Pt 20	Pt 23	Pt 26	Pt 29
cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33
Pt 3	Pt 6	Pt 9	Pt 12	Pt 15	Pt 18	Pt 21	Pt 24	Pt 27	Pt 30
cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31	cryo 31
Pt 3	Pt 6	Pt 9	Pt 12	Pt 15	Pt 18	Pt 21	Pt 24	Pt 27	Pt 30
cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32	cryo 32
Pt 3	Pt 6	Pt 9	Pt 12	Pt 15	Pt 18	Pt 21	Pt 24	Pt 27	Pt 30
cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33	cryo 33
Pt 31	Pt 31	Pt 31	Pt 32	Pt 32	Pt 32	Pt 33	Pt 33	Pt 33	X
cryo 31	cryo 32	cryo 33	cryo 31	cryo 32	cryo 33	cryo 31	cryo 32	cryo 33	

CALERIE EDTA 34 Tubes “LCBR to Aliquot”

EDTA 10mL Transfer Tube #34

Approximately 5.0mL sample volume. 1 tube per participant. 48 participants per box.

PT 1 cryo 34	PT 8 cryo 34	PT 15 cryo 34	PT 22 cryo 34	PT 29 cryo 34	PT 36 cryo 34	PT 43 cryo 34
PT 2 cryo 34	PT 9 cryo 34	PT 16 cryo 34	PT 23 cryo 34	PT 30 cryo 34	PT 37 cryo 34	PT 44 cryo 34
PT 3 cryo 34	PT 10 cryo 34	PT 17 cryo 34	PT 24 cryo 34	PT 31 cryo 34	PT 38 cryo 34	PT 45 cryo 34
PT 4 cryo 34	PT 11 cryo 34	PT 18 cryo 34	PT 25 cryo 34	PT 32 cryo 34	PT 39 cryo 34	PT 46 cryo 34
PT 5 cryo 34	PT 12 cryo 34	PT 19 cryo 34	PT 26 cryo 34	PT 33 cryo 34	PT 40 cryo 34	PT 47 cryo 34
PT 6 cryo 34	PT 13 cryo 34	PT 20 cryo 34	PT 27 cryo 34	PT 34 cryo 34	PT 41 cryo 34	PT 48 cryo 34
PT 7 cryo 34	PT 14 cryo 34	PT 21 cryo 34	PT 28 cryo 34	PT 35 cryo 34	PT 42 cryo 34	X

CALERIE Packed Red Cell Tubes for DNA Extraction

Packed Cells in 10mL Transfer Tubes (#35 & 37)

Approximately 5.0mL sample volume. 2 Tubes per participant. 24 participants per box.

PT 1 cryo 35	PT 4 cryo 37	PT 8 cryo 35	PT 11 cryo 37	PT 15 cryo 35	PT 18 cryo 37	PT 22 cryo 35
PT 1 cryo 37	PT 5 cryo 35	PT 8 cryo 37	PT 12 cryo 35	PT 15 cryo 37	PT 19 cryo 35	PT 22 cryo 37
PT 2 cryo 35	PT 5 cryo 37	PT 9 cryo 35	PT 12 cryo 37	PT 16 cryo 35	PT 19 cryo 37	PT 23 cryo 35
PT 2 cryo 37	PT 6 cryo 35	PT 9 cryo 37	PT 13 cryo 35	PT 16 cryo 37	PT 20 cryo 35	PT 23 cryo 37
PT 3 cryo 35	PT 6 cryo 37	PT 10 cryo 35	PT 13 cryo 37	PT 17 cryo 35	PT 20 cryo 37	PT 24 cryo 35
PT 3 cryo 37	PT 7 cryo 35	PT 10 cryo 37	PT 14 cryo 35	PT 17 cryo 37	PT 21 cryo 35	PT 24 cryo 37
PT 4 cryo 35	PT 7 cryo 37	PT 11 cryo 35	PT 14 cryo 37	PT 18 cryo 35	PT 21 cryo 37	X

CALERIE EDTA Tube 36 "LCBR to Aliquot"

EDTA 10mL EDTA Transfer Tube #36

Approximately 5.0mL sample volume; 1 tube per participant; 48 participants per box.

PT 1 cryo 36	PT 8 cryo 36	PT 15 cryo 36	PT 22 cryo 36	PT 29 cryo 36	PT 36 cryo 36	PT 43 cryo 36
PT 2 cryo 36	PT 9 cryo 36	PT 16 cryo 36	PT 23 cryo 36	PT 30 cryo 36	PT 37 cryo 36	PT 44 cryo 36
PT 3 cryo 36	PT 10 cryo 36	PT 17 cryo 36	PT 24 cryo 36	PT 31 cryo 36	PT 38 cryo 36	PT 45 cryo 36
PT 4 cryo 36	PT 11 cryo 36	PT 18 cryo 36	PT 25 cryo 36	PT 32 cryo 36	PT 39 cryo 36	PT 46 cryo 36
PT 5 cryo 36	PT 12 cryo 36	PT 19 cryo 36	PT 26 cryo 36	PT 33 cryo 36	PT 40 cryo 36	PT 47 cryo 36
PT 6 cryo 36	PT 13 cryo 36	PT 20 cryo 36	PT 27 cryo 36	PT 34 cryo 36	PT 41 cryo 36	PT 48 cryo 36
PT 7 cryo 36	PT 14 cryo 36	PT 21 cryo 36	PT 28 cryo 36	PT 35 cryo 36	PT 42 cryo 36	X

CALERIE PAXgene Tubes for RNA Isolation

PAXgene 2.5mL Collection Tubes (Tube #10 and 11)

2 Tubes per participant. 24 participants per box

PT 1 Tube 10	PT 4 Tube 11	PT 8 Tube 10	PT 11 Tube 11	PT 15 Tube 10	PT 18 Tube 11	PT 22 Tube 10
PT 1 Tube 11	PT 5 Tube 10	PT 8 Tube 11	PT 12 Tube 10	PT 15 Tube 11	PT 19 Tube 10	PT 22 Tube 11
PT 2 Tube 10	PT 5 Tube 11	PT 9 Tube 10	PT 12 Tube 11	PT 16 Tube 10	PT 19 Tube 11	PT 23 Tube 10
PT 2 Tube 11	PT 6 Tube 10	PT 9 Tube 11	PT 13 Tube 10	PT 16 Tube 11	PT 20 Tube 10	PT 23 Tube 11
PT 3 Tube 10	PT 6 Tube 11	PT 10 Tube 10	PT 13 Tube 11	PT 17 Tube 10	PT 20 Tube 11	PT 24 Tube 10
PT 3 Tube 11	PT 7 Tube 10	PT 10 Tube 11	PT 14 Tube 10	PT 17 Tube 11	PT 21 Tube 10	PT 24 Tube 11
PT 4 Tube 10	PT 7 Tube 11	PT 11 Tube 10	PT 14 Tube 11	PT 18 Tube 10	PT 21 Tube 11	X

CALERIE FAHC-Vitros Testing

Box Map for Tubes to FAHC: Serum - 4mL OGTT Tubes (Tubes# 38-41, 50 and 55)

Aliquots created during aliquotting

Ppt 1 Tube 50	Ppt 2 Tube 50	Ppt 3 Tube 50	Ppt 4 Tube 50	Ppt 5 Tube 50	Ppt 6 Tube 50	Ppt 7 Tube 50
Ppt 1 Tube 55	Ppt 2 Tube 55	Ppt 3 Tube 55	Ppt 4 Tube 55	Ppt 5 Tube 55	Ppt 6 Tube 55	Ppt 7 Tube 55
Ppt 1 Tube 38	Ppt 2 Tube 38	Ppt 3 Tube 38	Ppt 4 Tube 38	Ppt 5 Tube 38	Ppt 6 Tube 38	Ppt 7 Tube 38
Ppt 1 Tube 39	Ppt 2 Tube 39	Ppt 3 Tube 39	Ppt 4 Tube 39	Ppt 5 Tube 39	Ppt 6 Tube 39	Ppt 7 Tube 39
Ppt 1 Tube 40	Ppt 2 Tube 40	Ppt 3 Tube 40	Ppt 4 Tube 40	Ppt 5 Tube 40	Ppt 6 Tube 40	Ppt 7 Tube 40
Ppt 1 Tube 41	Ppt 2 Tube 41	Ppt 3 Tube 41	Ppt 4 Tube 41	Ppt 5 Tube 41	Ppt 6 Tube 41	Ppt 7 Tube 41
X	X	X	X	X	X	X

CALERIE Urine Repository Tubes

Plain Urine-10mL Transfer Tubes (#43-45) AND Boric Acid Urine 10mL Transfer Tubes (#46-48)
9.0mL sample volume. 6 tubes per participant. 8 participants per box.

PT 1 cryo 43	PT 2 cryo 43	PT 3 cryo 43	PT 4 cryo 43	PT 5 cryo 43	PT 6 cryo 43	PT 7 cryo 43
PT 1 cryo 44	PT 2 cryo 44	PT 3 cryo 44	PT 4 cryo 44	PT 5 cryo 44	PT 6 cryo 44	PT 7 cryo 44
PT 1 cryo 45	PT 2 cryo 45	PT 3 cryo 45	PT 4 cryo 45	PT 5 cryo 45	PT 6 cryo 45	PT 7 cryo 45
PT 1 cryo 46	PT 2 cryo 46	PT 3 cryo 46	PT 4 cryo 46	PT 5 cryo 46	PT 6 cryo 46	PT 7 cryo 46
PT 1 cryo 47	PT 2 cryo 47	PT 3 cryo 47	PT 4 cryo 47	PT 5 cryo 47	PT 6 cryo 47	PT 7 cryo 47
PT 1 cryo 48	PT 2 cryo 48	PT 3 cryo 48	PT 4 cryo 48	PT 5 cryo 48	PT 6 cryo 48	PT 7 cryo 48
PT 8 cryo 43	PT 8 cryo 44	PT 8 cryo 45	PT 8 cryo 46	PT 8 cryo 47	PT 8 cryo 48	X

CALERIE FAHC-Centaur Testing

LCBR prepared aliquots #51, 52, and 54
Use Box map as a guideline; will vary based on samples received.

cryo 51 (cortisol) ↓			cryo 54 (TSH,T3) ↓			male cryo 52 ↓		
PT 1	PT 8		PT 1	PT 8		PT 1		
cryo 51	cryo 51		cryo 54	cryo 54		male cryo 52		
PT 2	PT 9		PT 2	PT 9		PT 2		
cryo 51	cryo 51		cryo 54	cryo 54		male cryo 52		
PT 3	PT 10		PT 3	PT 10		PT 3		
cryo 51	cryo 51		cryo 54	cryo 54		male cryo 52		
PT 4	PT 11		PT 4	PT 11		PT 4		
cryo 51	cryo 51		cryo 54	cryo 54		male cryo 52		
PT 5	PT 12		PT 5	PT 12		PT 5		
cryo 51	cryo 51		cryo 54	cryo 54		male cryo 52		
PT 6	PT 13		PT 6	PT 13		PT 6		
cryo 51	cryo 51		cryo 54	cryo 54		male cryo 52		
PT 7	PT 14		PT 7	PT 14		PT 7		
cryo 51	cryo 51		cryo 54	cryo 54		male cryo 52		

CALERIE- 3M, 6M or 18M Serum Repository Cryovials

Serum - Red Top 1.5ml Cryo (Cryos# 03-06)

1.0ml sample volume. 4 cryos per participant. 25 participants per box.

Pt 1	Pt 3	Pt 6	Pt 8	Pt 11	Pt 13	Pt 16	Pt 18	Pt 21	Pt 23
cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05
Pt 1	Pt 3	Pt 6	Pt 8	Pt 11	Pt 13	Pt 16	Pt 18	Pt 21	Pt 23
cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06
Pt 1	Pt 4	Pt 6	Pt 9	Pt 11	Pt 14	Pt 16	Pt 19	Pt 21	Pt 24
cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03
Pt 1	Pt 4	Pt 6	Pt 9	Pt 11	Pt 14	Pt 16	Pt 19	Pt 21	Pt 24
cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04
Pt 2	Pt 4	Pt 7	Pt 9	Pt 12	Pt 14	Pt 17	Pt 19	Pt 22	Pt 24
cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05
Pt 2	Pt 4	Pt 7	Pt 9	Pt 12	Pt 14	Pt 17	Pt 19	Pt 22	Pt 24
cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06
Pt 2	Pt 5	Pt 7	Pt 10	Pt 12	Pt 15	Pt 17	Pt 20	Pt 22	Pt 25
cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03
Pt 2	Pt 5	Pt 7	Pt 10	Pt 12	Pt 15	Pt 17	Pt 20	Pt 22	Pt 25
cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04
Pt 3	Pt 5	Pt 8	Pt 10	Pt 13	Pt 15	Pt 18	Pt 20	Pt 23	Pt 25
cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05	cryo 03	cryo 05
Pt 3	Pt 5	Pt 8	Pt 10	Pt 13	Pt 15	Pt 18	Pt 20	Pt 23	Pt 25
cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06	cryo 04	cryo 06

CALERIE- 18 M Serum *FAHC* Cryo-Ab Response Testing

Serum - Red Top 1.5ml Cryo #03

1.0ml sample volume. 1 cryo per participant. 99 participants per box.

Pt 1	Pt 11	Pt 21	Pt 31	Pt 41	Pt 51	Pt 61	Pt 71	Pt 81	Pt 91
cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03
Pt 2	Pt 12	Pt 22	Pt 32	Pt 42	Pt 52	Pt 62	Pt 72	Pt 82	Pt 92
cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03
Pt 3	Pt 13	Pt 23	Pt 33	Pt 43	Pt 53	Pt 63	Pt 73	Pt 83	Pt 93
cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03
Pt 4	Pt 14	Pt 24	Pt 34	Pt 44	Pt 54	Pt 64	Pt 74	Pt 84	Pt 94
cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03
Pt 5	Pt 15	Pt 25	Pt 35	Pt 45	Pt 55	Pt 65	Pt 75	Pt 85	Pt 95
cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03
Pt 6	Pt 16	Pt 26	Pt 36	Pt 46	Pt 56	Pt 66	Pt 76	Pt 86	Pt 96
cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03
Pt 7	Pt 17	Pt 27	Pt 37	Pt 47	Pt 57	Pt 67	Pt 77	Pt 87	Pt 97
cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03
Pt 8	Pt 18	Pt 28	Pt 38	Pt 48	Pt 58	Pt 68	Pt 78	Pt 88	Pt 98
cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03
Pt 9	Pt 19	Pt 29	Pt 39	Pt 49	Pt 59	Pt 69	Pt 79	Pt 89	Pt 99
cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03
Pt 10	Pt 20	Pt 30	Pt 40	Pt 50	Pt 60	Pt 70	Pt 80	Pt 90	X
cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	cryo 03	

CALERIE 17M, 23M, OR Unscheduled Visits Serum Tubes

Serum-10mL Transfer Tube #02 rec'd for 17M, 23M, or Unscheduled CALERIE Visits

Approx. 2.0ml sample volume. 1 tubes per ppt. 48 ppts per box.

PT 1 tube 02	PT 8 tube 02	PT 15 tube 02	PT 22 tube 02	PT 29 tube 02	PT 36 tube 02	PT 43 tube 02
PT 2 tube 02	PT 9 tube 02	PT 16 tube 02	PT 23 tube 02	PT 30 tube 02	PT 37 tube 02	PT 44 tube 02
PT 3 tube 02	PT 10 tube 02	PT 17 tube 02	PT 24 tube 02	PT 31 tube 02	PT 38 tube 02	PT 45 tube 02
PT 4 tube 02	PT 11 tube 02	PT 18 tube 02	PT 25 tube 02	PT 32 tube 02	PT 39 tube 02	PT 46 tube 02
PT 5 tube 02	PT 12 tube 02	PT 19 tube 02	PT 26 tube 02	PT 33 tube 02	PT 40 tube 02	PT 47 tube 02
PT 6 tube 02	PT 13 tube 02	PT 20 tube 02	PT 27 tube 02	PT 34 tube 02	PT 41 tube 02	PT 48 tube 02
PT 7 tube 02	PT 14 tube 02	PT 21 tube 02	PT 28 tube 02	PT 35 tube 02	PT 42 tube 02	X

CALERIE Muscle IHC TISSUE (tissue cryo #02)

5.0mL vial with muscle tissue IHC samples

1 vial per ppt and 25 ppts per box in 5x5

PPT 1	PPT 6	PPT 11	PPT 16	PPT 21
PPT 2	PPT 7	PPT 12	PPT 17	PPT 22
PPT 3	PPT 8	PPT 13	PPT 18	PPT 23
PPT 4	PPT 9	PPT 14	PPT 19	PPT 24
PPT 5	PPT 10	PPT 15	PPT 20	PPT 25

*1 vial per ppt expected from all sites:
PBRC, Tufts and Wash U*

Adipose Tissue Cassette Biopsies

Bouin's Cassette sample #24 received from all 3 sites

n = 280 participants per storage box

40	80	120	160	200	240	280
39	79	119	159	199	239	279
38	78	118	158	198	238	278
37	77	117	157	197	237	277
36	76	116	156	196	236	276
35	75	115	155	195	235	275
34	74	114	154	194	234	274
33	73	113	153	193	233	273
32	72	112	152	192	232	272
31	71	111	151	191	231	271
30	70	110	150	190	230	270
29	69	109	149	189	229	269
28	68	108	148	188	228	268
27	67	107	147	187	227	267
26	66	106	146	186	226	266
25	65	105	145	185	225	265
24	64	104	144	184	224	264
23	63	103	143	183	223	263
22	62	102	142	182	222	262
21	61	101	141	181	221	261
20	60	100	140	180	220	260
19	59	99	139	179	219	259
18	58	98	138	178	218	258
17	57	97	137	177	217	257
16	56	96	136	176	216	256
15	55	95	135	175	215	255
14	54	94	134	174	214	254
13	53	93	133	173	213	253
12	52	92	132	172	212	252
11	51	91	131	171	211	251
10	50	90	130	170	210	250
9	49	89	129	169	209	249
8	48	88	128	168	208	248
7	47	87	127	167	207	247
6	46	86	126	166	206	246
5	45	85	125	165	205	245
4	44	84	124	164	204	244
3	43	83	123	163	203	243
2	42	82	122	162	202	242
1	41	81	121	161	201	241

CALERIE Muscle or Adipose TISSUE cryovials

2.0mL Corning cryovial with muscle or adipose tissue biopsies
9 x 9 grid 5 ppt max

Pt 1 cryo 01	Pt 1 cryo 23	Pt 2 cryo 04	Pt 2 cryo 26	Pt 3 cryo 06	Pt 3 cryo 28	Pt 4 cryo 08	Pt 4 cryo 30	Pt 5 cryo 22
Pt 1 cryo 03	Pt 1 cryo 25	Pt 2 cryo 05	Pt 2 cryo 27	Pt 3 cryo 07	Pt 3 cryo 29	Pt 4 cryo 21	Pt 5 cryo 01	Pt 5 cryo 23
Pt 1 cryo 04	Pt 1 cryo 26	Pt 2 cryo 06	Pt 2 cryo 28	Pt 3 cryo 08	Pt 3 cryo 30	Pt 4 cryo 22	Pt 5 cryo 03	Pt 5 cryo 25
Pt 1 cryo 05	Pt 1 cryo 27	Pt 2 cryo 07	Pt 2 cryo 29	Pt 3 cryo 21	Pt 4 cryo 01	Pt 4 cryo 23	Pt 5 cryo 04	Pt 5 cryo 26
Pt 1 cryo 06	Pt 1 cryo 28	Pt 2 cryo 08	Pt 2 cryo 30	Pt 3 cryo 22	Pt 4 cryo 03	Pt 4 cryo 25	Pt 5 cryo 05	Pt 5 cryo 27
Pt 1 cryo 07	Pt 1 cryo 29	Pt 1 cryo 21	Pt 3 cryo 01	Pt 3 cryo 23	Pt 4 cryo 04	Pt 4 cryo 26	Pt 5 cryo 06	Pt 5 cryo 28
Pt 1 cryo 08	Pt 1 cryo 30	Pt 1 cryo 22	Pt 3 cryo 03	Pt 3 cryo 25	Pt 4 cryo 05	Pt 4 cryo 27	Pt 5 cryo 07	Pt 5 cryo 29
Pt 1 cryo 21	Pt 2 cryo 01	Pt 2 cryo 23	Pt 3 cryo 04	Pt 3 cryo 26	Pt 4 cryo 06	Pt 4 cryo 28	Pt 5 cryo 08	Pt 5 cryo 30
Pt 1 cryo 22	Pt 2 cryo 03	Pt 2 cryo 25	Pt 3 cryo 05	Pt 3 cryo 27	Pt 4 cryo 07	Pt 4 cryo 29	Pt 5 cryo 21	x

2.0mL Biospy Cryos Expected

Adipose tissue

9 x 2.0ml Corning cryovials *All 3 sites*
(#21-23 and #25-30)

Muscle Tissue

3 x 2.0mL Corning cryovials *PBRC and Wash U*
#01,03,08

7 x 2.0mL Corning cryovials *Tufts*
#01-08

B.2 LCBR ALIQUOT BOXES

CALERIE LCBR Serum Assay Cryovials

Serum - Red Top 0.5 or 1.5ml Cryo (Cryos# 52 (females only) and #65-70).
10 participants per box.

Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
cryo 14	cryo 14	cryo 14	cryo 14	cryo 14	cryo 14	cryo 14	cryo 14	cryo 14	cryo 14
Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
cryo 15	cryo 15	cryo 15	cryo 15	cryo 15	cryo 15	cryo 15	cryo 15	cryo 15	cryo 15
Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
cryo 16	cryo 16	cryo 16	cryo 16	cryo 16	cryo 16	cryo 16	cryo 16	cryo 16	cryo 16
Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
cryo 17	cryo 17	cryo 17	cryo 17	cryo 17	cryo 17	cryo 17	cryo 17	cryo 17	cryo 17
Pt 1	Pt 2	Pt 1	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
cryo 52	cryo 52	cryo 52	cryo 52	cryo 52	cryo 52	cryo 52	cryo 52	cryo 52	cryo 52
Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
cryo 65	cryo 65	cryo 65	cryo 65	cryo 65	cryo 65	cryo 65	cryo 65	cryo 65	cryo 65
Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
cryo 66	cryo 66	cryo 66	cryo 66	cryo 66	cryo 66	cryo 66	cryo 66	cryo 66	cryo 66
Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
cryo 67	cryo 67	cryo 67	cryo 67	cryo 67	cryo 67	cryo 67	cryo 67	cryo 67	cryo 67
Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
cryo 68	cryo 68	cryo 68	cryo 68	cryo 68	cryo 68	cryo 68	cryo 68	cryo 68	cryo 68
Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9	Pt 10
cryo 70	cryo 70	cryo 70	cryo 70	cryo 70	cryo 70	cryo 70	cryo 70	cryo 70	cryo 70

CALERIE LCBR EDTA Assay Cryovials

EDTA Purple Top 1.5mL Cryos (#88)

0.5mL sample volume 1 cryo per participant. 99 participants per box

Pt 1	Pt 11	Pt 21	Pt 31	Pt 41	Pt 51	Pt 61	Pt 71	Pt 81	Pt 91
cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88
Pt 2	Pt 12	Pt 22	Pt 32	Pt 42	Pt 52	Pt 62	Pt 72	Pt 82	Pt 92
cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88
Pt 3	Pt 13	Pt 23	Pt 33	Pt 43	Pt 53	Pt 63	Pt 73	Pt 83	Pt 93
cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88
Pt 4	Pt 14	Pt 24	Pt 34	Pt 44	Pt 54	Pt 64	Pt 74	Pt 84	Pt 94
cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88
Pt 5	Pt 15	Pt 25	Pt 35	Pt 45	Pt 55	Pt 65	Pt 75	Pt 85	Pt 95
cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88
Pt 6	Pt 16	Pt 26	Pt 36	Pt 46	Pt 56	Pt 66	Pt 76	Pt 86	Pt 96
cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88
Pt 7	Pt 17	Pt 27	Pt 37	Pt 47	Pt 57	Pt 67	Pt 77	Pt 87	Pt 97
cryo 88	18	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88
Pt 8	Pt 18	Pt 28	Pt 38	Pt 48	Pt 58	Pt 68	Pt 78	Pt 88	Pt 98
cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88
Pt 9	Pt 19	Pt 29	Pt 39	Pt 49	Pt 59	Pt 69	Pt 79	Pt 89	Pt 99
cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88
Pt 10	Pt 20	Pt 30	Pt 40	Pt 50	Pt 60	Pt 70	Pt 80	Pt 90	X
cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	cryo 88	

CALERIE LCBR Repository B- Annual Visit Repository for 1 Participant

Serum Red top 0.5mL or 1.5mL Cryovials (#53, 57-62,69,71-74,76-83)

EDTA Purple Top 0.5mL Cryovials (#84-87 and 89-97)

BL cryo 53	BL cryo 73	BL cryo 84	BL cryo 95	12M cryo 69	12M cryo 81	12M cryo 92	24M cryo 60	24M cryo 78
BL cryo 57	BL cryo 74	BL cryo 85	BL cryo 96	12M cryo 71	12M cryo 82	12M cryo 93	24M cryo 61	24M cryo 79
BL cryo 58	BL cryo 76	BL cryo 86	BL cryo 97	12M cryo 72	12M cryo 83	12M cryo 94	24M cryo 62	24M cryo 80
BL cryo 59	BL cryo 77	BL cryo 87	12M cryo 53	12M cryo 73	12M cryo 84	12M cryo 95	24M cryo 69	24M cryo 81
BL cryo 60	BL cryo 78	BL cryo 89	12M cryo 57	12M cryo 74	12M cryo 85	12M cryo 96	24M cryo 71	24M cryo 82
BL cryo 61	BL cryo 79	BL cryo 90	12M cryo 58	12M cryo 76	12M cryo 86	12M cryo 97	24M cryo 72	24M cryo 83
BL cryo 62	BL cryo 80	BL cryo 91	12M cryo 59	12M cryo 77	12M cryo 87	24M cryo 53	24M cryo 73	24M cryo 84
BL cryo 69	BL cryo 81	BL cryo 92	12M cryo 60	12M cryo 78	12M cryo 89	24M cryo 57	24M cryo 74	24M cryo 85
BL cryo 71	BL cryo 82	BL cryo 93	12M cryo 61	12M cryo 79	12M cryo 90	24M cryo 58	24M cryo 76	24M cryo 86
BL cryo 72	BL cryo 83	BL cryo 94	12M cryo 62	12M cryo 80	12M cryo 91	24M cryo 59	24M cryo 77	24M cryo 87

CALERIE OGTT Serum Repository Cryovials

Serum Red Top 1.5mL Cryos (#18,19,98,99);

1.0ml sample volume. 4 cryos per participants. 25 participants per box.

Pt 1 cryo 18	Pt 3 cryo 98	Pt 6 cryo 18	Pt 8 cryo 98	Pt 11 cryo 18	Pt 13 cryo 98	Pt 16 cryo 18	Pt 18 cryo 98	Pt 21 cryo 18	Pt 23 cryo 98
Pt 1 cryo 19	Pt 3 cryo 99	Pt 6 cryo 19	Pt 8 cryo 99	Pt 11 cryo 19	Pt 13 cryo 99	Pt 16 cryo 19	Pt 18 cryo 99	Pt 21 cryo 19	Pt 23 cryo 99
Pt 1 cryo 98	Pt 4 cryo 18	Pt 6 cryo 98	Pt 9 cryo 18	Pt 11 cryo 98	Pt 14 cryo 18	Pt 16 cryo 98	Pt 19 cryo 18	Pt 21 cryo 98	Pt 24 cryo 18
Pt 1 cryo 99	Pt 4 cryo 19	Pt 6 cryo 99	Pt 9 cryo 19	Pt 11 cryo 99	Pt 14 cryo 19	Pt 16 cryo 99	Pt 19 cryo 19	Pt 21 cryo 99	Pt 24 cryo 19
Pt 2 cryo 18	Pt 4 cryo 98	Pt 7 cryo 18	Pt 9 cryo 98	Pt 12 cryo 18	Pt 14 cryo 98	Pt 17 cryo 18	Pt 19 cryo 98	Pt 22 cryo 18	Pt 24 cryo 98
Pt 2 cryo 19	Pt 4 cryo 99	Pt 7 cryo 19	Pt 9 cryo 99	Pt 12 cryo 19	Pt 14 cryo 99	Pt 17 cryo 19	Pt 19 cryo 99	Pt 22 cryo 19	Pt 24 cryo 99
Pt 2 cryo 98	Pt 5 cryo 18	Pt 7 cryo 98	Pt 10 cryo 18	Pt 12 cryo 98	Pt 15 cryo 18	Pt 17 cryo 98	Pt 20 cryo 18	Pt 22 cryo 98	Pt 25 cryo 18
Pt 2 cryo 99	Pt 5 cryo 19	Pt 7 cryo 99	Pt 10 cryo 19	Pt 12 cryo 99	Pt 15 cryo 19	Pt 17 cryo 99	Pt 20 cryo 19	Pt 22 cryo 99	Pt 25 cryo 19
Pt 3 cryo 18	Pt 5 cryo 98	Pt 8 cryo 18	Pt 10 cryo 98	Pt 13 cryo 18	Pt 15 cryo 98	Pt 18 cryo 18	Pt 20 cryo 98	Pt 23 cryo 18	Pt 25 cryo 98
Pt 3 cryo 19	Pt 5 cryo 99	Pt 8 cryo 19	Pt 10 cryo 99	Pt 13 cryo 19	Pt 15 cryo 99	Pt 18 cryo 19	Pt 20 cryo 99	Pt 23 cryo 19	Pt 25 cryo 99

CALERIE FAHC-Immulinite Testing

Serum 5mL 12 x 75mm Tube (356)

1 Tube per participant, 49 participants per box.

PT 1 cryo 56	PT 8 cryo 56	PT 15 cryo 56	PT 22 cryo 56	PT 29 cryo 56	PT 36 cryo 56	PT 43 cryo 56
PT 2 cryo 56	PT 9 cryo 56	PT 16 cryo 56	PT 23 cryo 56	PT 30 cryo 56	PT 37 cryo 56	PT 44 cryo 56
PT 3 cryo 56	PT 10 cryo 56	PT 17 cryo 56	PT 24 cryo 56	PT 31 cryo 56	PT 38 cryo 56	PT 45 cryo 56
PT 4 cryo 56	PT 11 cryo 56	PT 18 cryo 56	PT 25 cryo 56	PT 32 cryo 56	PT 39 cryo 56	PT 46 cryo 56
PT 5 cryo 56	PT 12 cryo 56	PT 19 cryo 56	PT 26 cryo 56	PT 33 cryo 56	PT 40 cryo 56	PT 47 cryo 56
PT 6 cryo 56	PT 13 cryo 56	PT 20 cryo 56	PT 27 cryo 56	PT 34 cryo 56	PT 41 cryo 56	PT 48 cryo 56
PT 7 cryo 56	PT 14 cryo 56	PT 21 cryo 56	PT 28 cryo 56	PT 35 cryo 56	PT 42 cryo 56	PT 49 cryo 56

*Box Maps for FAHC –Vitros Testing and FAHC –Centaur Testing are used again at aliquotting.
See appendix B.1 for box maps.*

APPENDIX C. CALERIE LCBR ALIQUOTTING GUIDE

CALERIE ALIQUOTTING SCHEME BL,12M, 24M Visits

updated 4/14/09 RHB

*NOTE: ALLOW all Serum and EDTA transfer tubes to completely thaw to room temperature in 37C water bath prior to aliquotting; Approx 5-7 minutes total per rack

SERUM

1. Thaw Transfer Tubes 25, 26, 27 & 28 to Room Temp. *

2. Pool Transfer Tubes 25, 26, 27 & 28 into a 50mL conical tube

3. Gently INVERT Pooled Serum 15 times

4. Aliquot FAHC Testing Cryovials First

Cryo/Tube#	Aliquot Volume	Tube/Cryo size	FAHC Box
50	600	false bottom	FAHC/vitros (LPR)
51	300	12 x 75 (5mL)	FAHC/Centaur (CORT)
52 (males only)	500	12 x 75 (5mL)	FAHC/Centaur (male only)
54	500	12 x 75 (5mL)	FAHC/Centaur (TSH, T3)
55	200	false bottom	FAHC/Vitros (SGL)
56	500	12 x 75 (5mL)	FAHC/Immulin e /DHES)
75	500	12 x 75 (5mL)	FAHC/Mayo (CPEP)

5. Aliquot LCBR Testing Cryovials Second

Cryo/Tube#	Aliquot Volume	Tube/Cryo size	FAHC Box
52 (females only)	200	0.5mL cryo (red)	LCBR Serum Assay
65	500	0.5mL cryo (red)	LCBR Serum Assay
66	500	0.5mL cryo (red)	LCBR Serum Assay
67	500	0.5mL cryo (red)	LCBR Serum Assay
68	500	0.5mL cryo (red)	LCBR Serum Assay
70	1000	1.5mL cryo (red)	LCBR Serum Assay

6. Aliquot Repository Cryovials Last

Cryo/Tube #	Aliquot Volume	Tube/Cryo size	Repository Box
53	500	0.5mL cryo (red)	LCBR Repos B
57	1200	1.5mL cryo (red)	LCBR Repos B
58	500	0.5mL cryo (red)	LCBR Repos B
59	500	0.5mL cryo (red)	LCBR Repos B
60	500	0.5mL cryo (red)	LCBR Repos B
61	500	0.5mL cryo (red)	LCBR Repos B
62	500	0.5mL cryo (red)	LCBR Repos B
69	500	0.5mL cryo (red)	LCBR Repos B
71	500	0.5mL cryo (red)	LCBR Repos B
72	500	0.5mL cryo (red)	LCBR Repos B
73	500	0.5mL cryo (red)	LCBR Repos B
74	500	0.5mL cryo (red)	LCBR Repos B
76	500	0.5mL cryo (red)	LCBR Repos B
77	500	0.5mL cryo (red)	LCBR Repos B
78	500	0.5mL cryo (red)	LCBR Repos B
79	500	0.5mL cryo (red)	LCBR Repos B
80	500	0.5mL cryo (red)	LCBR Repos B
81	500	0.5mL cryo (red)	LCBR Repos B
82	500	0.5mL cryo (red)	LCBR Repos B
83	500	0.5mL cryo (red)	LCBR Repos B

7. Thaw OGTT Tubes 38-41 to Room Temp*

8. Gently INVERT Each OGTT tube 15 times

9. Aliquot OGTT tubes as follows (remember to change tips between OGTT tubes!)

OGTT Tube #	Cryo/Tube #	Aliquot Volume	Tube/Cryo size	Repository Box
38	38	200	original 4ml false	FAHC/Vitros (SGL)
	08	500	12 x 75 (5mL)	FAHC/Mayo (CPEP)
	14	200	0.5mL cryo (red)	LCBR Assay (INS)
	18	1000	1.5mL cryo (red)	OGTT Repository
39	39	200	original 4ml false	FAHC/Vitros (SGL)
	09	500	12 x 75 (5mL)	FAHC/Mayo (CPEP)
	15	200	0.5mL cryo (red)	LCBR Assay (INS)
	19	1000	1.5mL cryo (red)	OGTT Repository
40	40	200	original 4ml false bottom	FAHC/Vitros (SGL)
	12	500	12 x 75 (5mL)	FAHC/Mayo (CPEP)
	16	200	0.5mL cryo (red)	LCBR Assay (INS)
	98	1000	1.5mL cryo (red)	OGTT Repository
41	41	200	original 4ml false	FAHC/Vitros (SGL)
	13	500	12 x 75 (5mL)	FAHC/Mayo (CPEP)
	17	200	0.5mL cryo (red)	LCBR Assay (INS)
	99	1000	1.5mL cryo (red)	OGTT Repository

EDTA

1. Thoroughly thaw EDTA transfer tube 34 to Room
2. Gently INVERT EDTA Tube # 34 15 times
3. Aliquot cryovials 84-87

Transfer Tube #	Cryo/Tube #	Aliquot Volume	Tube/Cryo size	Repository Box
34	84	500	1.5mL (purple cap)	LCBR Repos B
	85	500	1.5mL (purple cap)	LCBR Repos B
	86	500	1.5mL (purple cap)	LCBR Repos B
	87	500	1.5mL (purple cap)	LCBR Repos B

4. Thoroughly thaw EDTA transfer tube # 36 to Room Temp. *
5. Gently INVERT EDTA Tube # 36 15 times
6. Aliquot cryovials 88-97 (remember to change pipette tips!)

Transfer Tube #	Cryo/Tube #	Aliquot Volume	Tube/Cryo size	Repository Box
36	88	500	0.5mL (purple cap)	LCBR Repos B
	89	500	0.5mL (purple cap)	LCBR Repos B
	90	500	0.5mL (purple cap)	LCBR Repos B
	91	500	0.5mL (purple cap)	LCBR Repos B
	92	500	0.5mL (purple cap)	LCBR Repos B
	93	500	0.5mL (purple cap)	LCBR Repos B
	94	500	0.5mL (purple cap)	LCBR Repos B
	95	500	0.5mL (purple cap)	LCBR Repos B
	96	500	0.5mL (purple cap)	LCBR Repos B
	97	500	1.5mL (purple cap)	LCBR Repos B

APPENDIX D. CALERIE Core Lab Supply Ordering Information

TUBES AND CRYOS

0.5mL free-standing cryos

Fisher order #02-681-333

500/pk, 10pk/case

1.5mL free-standing cryos

Fisher order 02-681-338

500/pk, 10pk/case

Red cryo caps

Fisher order #02-681-361

500/pk, 10pk/case

Purple cryo caps

Fisher order #02-681-366

500/pk, 10pk/case

Blue cryo caps

Fisher order #02-681-348

500/pk, 10pk/case

5mL 12/75mm polypropylene tubes

VWR: 60818-430

case of 1000 bulk

Red tube plug-type caps

VWR: 60819-036

pack of 1000

4mL OGTT Tubes

Fisher order# NC9970600

Sarstedt: 62.611

500/pk

10mL Transfer Tubes

Fisher order# 05-680-00

Simport: T550-10ATPR

1000/case

SHIPPING**Smaller Insulated Shippers:**

Fisher order #03-530-17

SCA THERMOSAFE No.:355

4 per case

Larger Insulated Shippers:

Fisher order #03-530-32

SCA THERMOSAFE No.:398

2 per case

Saf-T-Pak 15/30C PCM Packs

Fisher order #NC9122846

STP-317 Saf-T-Pak 15/30C Packs

8 packs and 4 sleeves per case

Small cardboard mailers (no cooler)

Fisher order #11-676-14

SCA THERMOSAFE No.:318

12 per case

TISSUE CONTAINERS/CRYOS**2.0mL Corning Tubes**

Fisher order#03-374-21

Corning: 430488

500/case

5mL Nalgene sample vials

Fisher order# 03-388A

Nalgene: 6250-0005

12/pk, 12 pks/case

4oz sample containers

Fisher order# 02-540-19

Corning: 1730 4L

200/case

Richard-Allan HistoScreen Cassettes

Fisher order #22-045344

Thermo Scientific # 1000-WH

250/pk, 4 pk/case

BLOOD COLLECTION TUBES *Order in 100/pk or 10pk/case*

10mL Serum

Fisher order# 02-685-112
BD: 367820

4mL Serum

Fisher order# 02-685-111
BD: 367812

2.7mL Citrate

Fisher order# 02-683-172
BD: 363083

10mL EDTA

Fisher order# 02-657-32
BD: 366643

6.0mL EDTA

Fisher order# 02-683-99D
BD: 367863

2.5mL PAXgene

VWR part # 77776-026
BD: 762165

APPENDIX E. CALERIE DATA REVIEW AND APPROVAL Signature Templates

CALERIE Study Data Review/Transmit Approval

PI or Coordinator Signature

Date

CALERIE Quality Control Data Approval

PI or Coordinator Signature

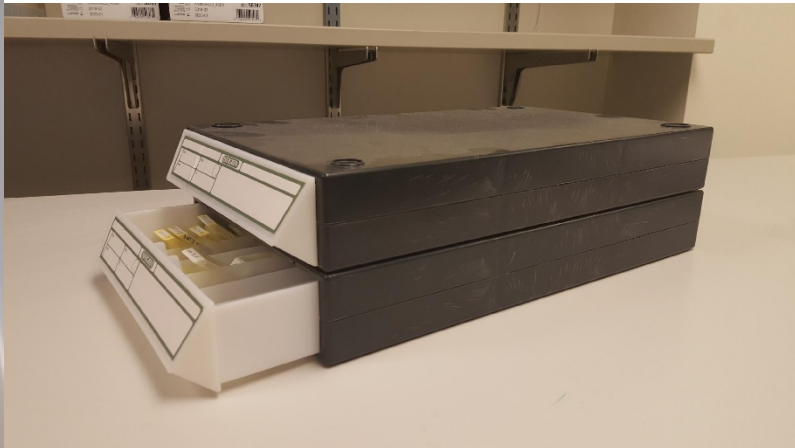
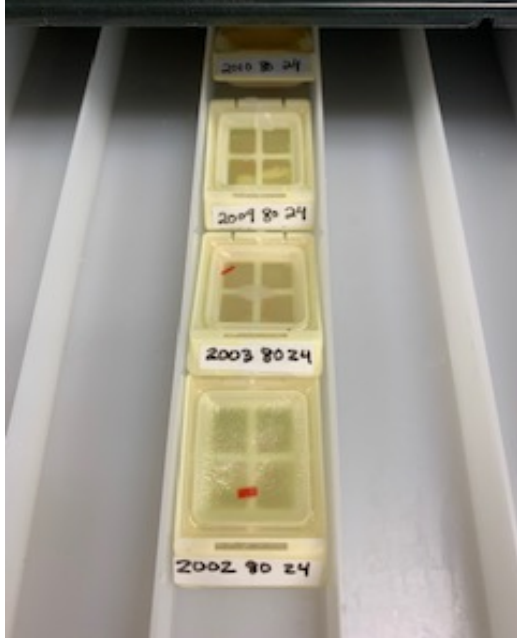
Date

CALERIE Study Data Transmit

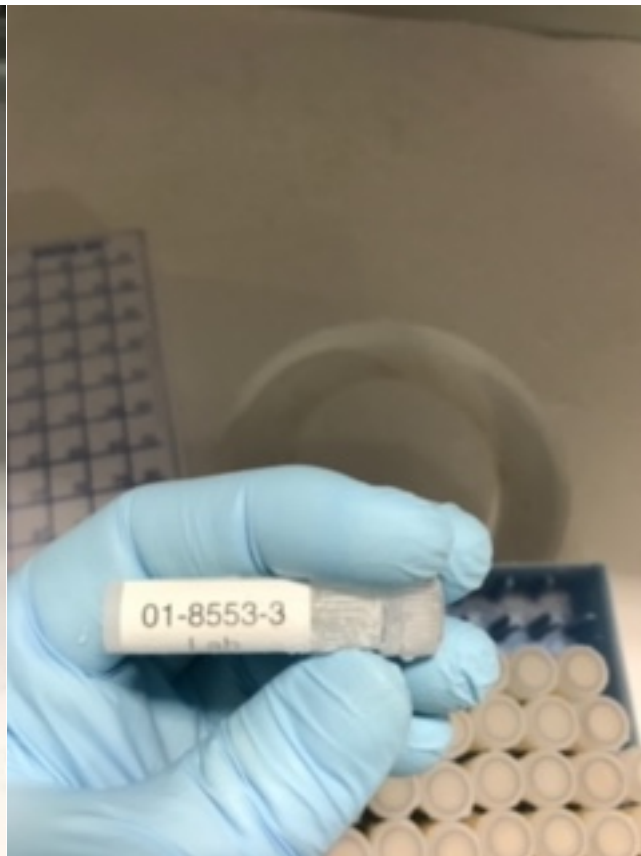
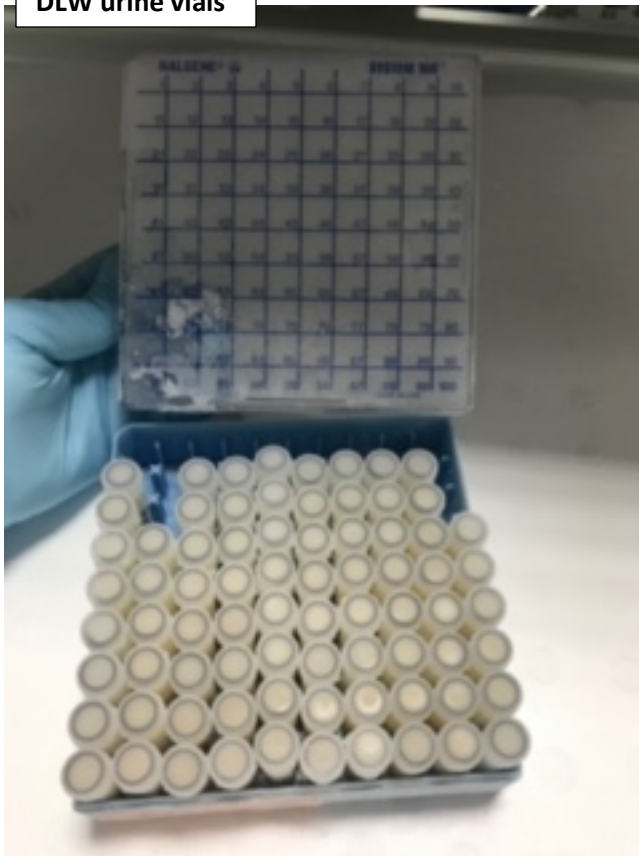
Project Manager Signature

Date of Transmit

Adipose Tissue Cassettes



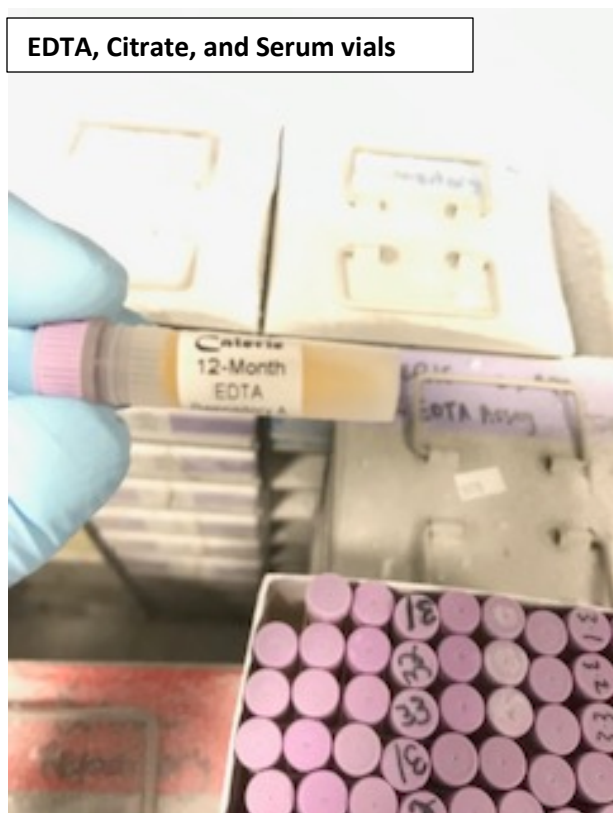
DLW urine vials



DNA and RNA vials



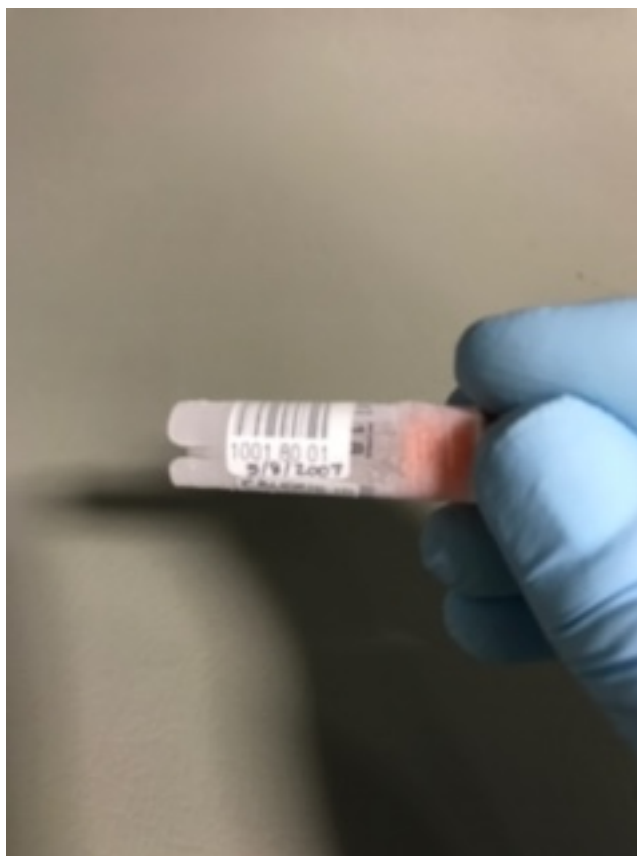
EDTA, Citrate, and Serum vials



Muscle IHC vials



Tissue archive vials



Urine Tubes

